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## A Review of the Present Status of Barbiturate Regulation*

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### INTRODUCTION

THE Committee on Legislation of the AMERICAN PHARMACEUTICAL ASSOCIATION has inaugurated a program dealing with the problems arising from the misuse of barbiturates. As preliminary steps in the development of this program, an opinion survey has been conducted among state pharmacy officials and practicing pharmacists on proper methods of control, a detailed study of the existing laws dealing with this subject has been made, and a Conference on Barbiturates[1] was held at the A. PH. A. Headquarters in Washington on October 12, 1945. This meeting was attended by representatives of the American Medical Association, American Dental Association, American Hospital Association, U. S. Public Health Service, U. S. Bureau of Narcotics and the U. S. Food and Drug Administration, together with state law enforcement officials and representatives of retail, wholesale and manufacturing groups in pharmacy.

This meeting was characterized by free and detailed discussion of the various aspects of the problem and resulted in a crystallization of the opinions and experiences of the professions and law enforcement officials which will be of great value to the Committee on Legislation in planning the course of action to be taken. It was generally agreed that any proposed legislation must go beyond the control of "over the counter" sales of these dangerous drugs by pharmacists and control the dispensing physician, restrict the refilling of prescriptions, prohibit distribution to illegitimate channels by irresponsible manufacturers and wholesalers and make the unlawful possession of these drugs a criminal offense. It was further agreed that in addition to a legislative program it will be necessary to follow this with an educational program which will lead to

* Presented in summary to the Conference on the Regulation of Use and Distribution of Barbiturates called by the AMERICAN PHARMACEUTICAL ASSOCIATION at Washington, D. C., October 12, 1945. Elaboration of the original article to include the details of each state law dealing with barbiturates was completed by Dr. Joseph B. Burt, on leave from the University of Nebraska, under a grant from the George and Lillian Judisch Fund and the A. PH. A. General Expense Budget.

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[1] For a report on this meeting, see THIS JOURNAL, Practical Pharmacy Edition, **7**, 356–58(1945).

the education of legislators on the need for adequate enforcement funds and procedures; education of pharmacists on the potential danger of these drugs and the need for compliance with reasonable regulation; education of physicians on the need for conservative prescribing of barbiturates; and education of laymen on the risks involved in the use of these drugs without proper medical supervision.

The report which follows is a summary of the present laws dealing with the regulation and distribution of barbiturates and other hypnotic drugs and represents a part of the study which is being made under the auspices of the Committee on Legislation. A somewhat similar survey,[2] prepared by the Bureau of Legal Medicine and Legislation of the American Medical Association, was published in 1940. Although this summary was of value, particularly in checking with information obtained from the laws themselves, it was not always in agreement with our findings and for that reason we have based our report upon information obtained directly from the laws rather than depending upon this or other summaries which were available.

## EXISTING LAWS

As of October 1, 1945, thirty-five states, possessions or district had laws in force which either directly or indirectly control, or could be utilized as a basis for control of the distribution of barbiturates alone or of barbiturates and other hypnotic drugs. The District of Columbia and six states have also issued regulations which are supplementary to existing laws. In addition, four states which do not have barbiturate or similar laws have issued through appropriate agencies of their state governments regulations which exercise control over the retail distribution of these drugs.

Since two possessions (Alaska and Puerto Rico) and the District of Columbia are included in the total given above, 36 states (32 by statute, 4 by regulation) are accounted for. This leaves 12 states that have no laws or regulations pertaining to this subject. These are as follows:

| | |
|---|---|
| Arizona | New Mexico |
| Idaho | Ohio |
| Illinois | South Dakota |
| Iowa | Texas |
| Kentucky | Wisconsin |
| Massachusetts | Wyoming |

[2] "Regulation of the Sale of Barbiturates by Statutes," *J. Am. Med. Assoc.*, **114**, 2029–36(1940).

Although city laws and ordinances have in several instances antedated state laws or regulations on barbiturates, the first state law dealing specifically with barbiturates was that of California, enacted in 1929. This law has since been repealed and replaced by a new law which was approved July 10, 1945. A list of the states, possessions or district having laws either of a special or general type, arranged in the order of their adoption, is shown in Table I.

TABLE I—LAWS REGULATING THE DISTRIBUTION OF BARBITURATES AND OTHER HYPNOTIC DRUGS

(Arranged in Order of Their Adoption)

I. Special Laws Covering Barbiturates with or without Other Hypnotic Drugs:
- 1929—California
- 1930—Virginia
- 1933—New Jersey, Maine, Oklahoma[a]
- 1935—Alabama, Arkansas, Colorado, Maryland, Nebraska, Oregon, Pennsylvania, West Virginia
- 1937—Rhode Island, South Carolina
- 1939—Delaware, Georgia, Minnesota, Tennessee, Washington
- 1940—Mississippi
- 1941—Alaska, Puerto Rico
- 1943—Michigan
- 1945—New York

II. General Laws
- 1921—New Hampshire[b]
- 1931—North Carolina
- 1936—Louisiana
- 1938—District of Columbia
- 1939—Connecticut, Florida, Indiana, Nevada, Vermont[c]
- 1943—Missouri[d]

[a] Law of 1933 has been repealed and superseded by the law of 1945.

[b] A general pharmacy law limiting the sale of all potent drugs, defined as those having an average adult dose of less than 60 gr., to registered pharmacists and assistant pharmacists.

[c] The State Narcotic Law of Vermont was amended in 1939 to include barbiturates.

[d] This is the State Uniform Food, Drug and Cosmetic Act. Although no direct reference is made to barbiturates, the law prohibits the sale, except on prescription, of drugs which are "dangerous to health." The absence of a regulation by the State Board of Health declaring barbiturates "dangerous" indicates that the law is not being used for this purpose, although it appears that authority to promulgate such a regulation is granted to this agency by the law.

## TYPES OF LAWS

The laws of the 32 states, Alaska, Puerto Rico and the District of Columbia, follow no regular pattern, either with regard to general structure or the drugs which are covered. Twenty-five laws are specific in nature, covering barbiturates alone or either barbiturates and other hypnotic drugs or barbiturates and other "dangerous" drugs, regardless of therapeutic action. Only eight laws are limited to the control of barbiturates exclusively. Ten additional special laws cover only barbiturates and other hypnotic or somnifacient drugs. The remaining seven special laws are distributed among five different classifications, based upon the drugs which are covered.

TABLE II—TYPES OF LAWS

I. States having special laws covering barbiturates (either including or excluding other hypnotics and/or other drugs)

1. Alaska
2. Alabama
3. Arkansas
4. California
5. Colorado[a]
6. Delaware
7. Georgia
8. Maine
9. Maryland
10. Michigan[a]
11. Minnesota
12. Mississippi
13. Nebraska
14. New Jersey[a]
15. New York
16. Oklahoma
17. Oregon
18. Puerto Rico
19. Pennsylvania
20. Rhode Island
21. South Carolina
22. Tennessee
23. Virginia
24. Washington
25. West Virginia

A. Laws Covering Barbiturates Only

1. Arkansas
2. Maine
3. Minnesota
4. Mississippi
5. Oregon
6. South Carolina
7. Tennessee
8. West Virginia[b]

B. Laws Covering Barbiturates and Other Hypnotic or Somnifacient Drugs Only

1. Alabama
2. Colorado[a]
3. Delaware
4. Maryland
5. Michigan[a]
6. Nebraska
7. New Jersey[a]
8. New York
9. Oklahoma
10. Rhode Island

C. Laws Covering Barbiturates and Sulfa Drugs Only

1. Georgia
2. Puerto Rico
3. Washington

D. Laws Covering Barbiturates, Sulfa Drugs and Hormones

1. Virginia

E. Laws Covering Barbiturates, Other Hypnotics (Chloral Hydrate) and Sulfa Drugs

1. Alaska

F. Laws Covering Barbiturates, Other Hypnotic, Analgesic and Body Weight Reducing Drugs

1. Pennsylvania[a]

G. Laws Covering Barbiturates and Other Hypnotics, Aminopyrine and Derivatives, Amphetamine and Desoxyephedrine, Diethylstilbestrol, Oils of Rue, Croton, and Tansy, Sulfa Drugs and Thyroid

1. California

II. General Laws

1. Connecticut
2. District of Columbia[a]
3. Florida
4. Indiana[a]
5. Louisiana[a]
6. Missouri
7. Nevada
8. New Hampshire
9. North Carolina
10. Vermont

A. "Uniform" Food, Drug and Cosmetic Laws

1. With a Specific Clause Mentioning Barbiturates and Other Drugs

1. Connecticut
2. Florida
3. Nevada
4. North Carolina

2. Without a Specific Clause Mentioning Barbiturates and Other Drugs

1. District of Columbia[a]
2. Indiana[a]
3. Missouri

B. State Food, Drug and Cosmetic Laws Enacted Prior to 1938

1. Louisiana[a]

C. State Narcotic Laws (as Amended to Include Barbiturates and Other Drugs)

1. Vermont

D. General Law Covering Potent Drugs

1. New Hampshire

III. States Having Regulations Only

1. Kansas
2. Montana
3. North Dakota
4. Utah

[a] Regulations have also been issued.
[b] West Virginia has separate subsections of its Narcotic Drug Act for barbiturates and for chloral hydrate.

Ten laws are of a general type such as the Uniform State Food, Drug and Cosmetic Laws, or the State Narcotic Law.

A classification of the thirty-five laws, according to type, is shown in Table II.

## COVERAGE OF THE LAWS

As indicated above, the lists of drugs covered by the individual laws show such wide divergence that their classification is extremely difficult. It is only by using rather broad classifications with reference to groups of drugs that a systematic arrangement can be devised, and even this requires 19 separate classifications and fails to include the specific drugs included in the laws or regulations of two of the states and the District of Columbia.

The classification follows:

1. Barbiturates only:
   Arkansas, Maine, Oregon, South Carolina, Tennessee
2. Barbiturates and chloral hydrate and derivatives:[3]
   West Virginia[3]
3. Barbiturates, chloral hydrate and paraldehyde:
   Michigan
4. Barbital, trional, sulfonal, tetronal, paraldehyde, chloral, chloral hydrate and derivatives:
   Alabama
5. Barbital, trional, sulfonal, chloral, chloral hydrate, carbromal and derivatives:
   Colorado

[3] Contained in separate subsections of the State Uniform Narcotic Act.

6. Barbital, trional, sulfonal, tetronal, chloral, chloral hydrate, chlorbutanol and derivatives:
   Delaware
   Maryland
   Rhode Island
7. Barbital, trional, sulfonal, tetronal, carbromal, paraldehyde, chloral, chloral hydrate, chlorobutanol and derivatives:
   Nebraska
   New Jersey
   New York
8. Barbiturates, Isonipecaine and benzedrine:
   Oklahoma
9. Barbiturates and sulfa drugs:
   Georgia
   Puerto Rico
   Washington
10. Barbiturates, chloral hydrate and derivatives and sulfa drugs:
    Alaska
    Minnesota
    Mississippi
11. Barbiturates, sulfa drugs and hormones:
    Virginia
12. Ami opyrine, barbituric acid, cinchophen, dinitrophenol, sulfanilamide and derivatives:
    Connecticut[4]
    Nevada[4]
    North Carolina[4]
13. Ammopyrine, barbituric acid, cinchophen, dinitrophenol, penicillin, sulfanilamide and derivatives:
    Florida[4]
14. Barbiturates, trional, sulfonal, tetronal, chloral, chloral hydrate, cinchophen (atophan), atoquinol, dinitrophenol, dinitrocresol, amphetamine (benzedrine) and thyroid:
    Pennsylvania
15. Narcotic drugs, barbiturates, cinchophen and sulfa drugs:
    Vermont[5]
16. Any drug dangerous to health when used in the dosage, or with the frequence or duration prescribed, recommended or suggested in the labeling thereof:
    Missouri[6]
17. Nonspecific drug list; (all substances having an average adult dose of 60 gr. or minims or less):
    New Hampshire
18. Special list established by regulation:
    Indiana[6]
    Louisiana
    District of Columbia[6]
19. Any hypnotic drug; aminopyrine and derivatives; amphetamine, desoxyephedrine and derivatives; cinchophen, neocinchophen and derivatives; diethylstilbestrol, or compounds or mixtures thereof; ergot, cotton root and derivatives; oils of croton, rue or savin and derivatives; sulfanilamide and derivatives:
    California

[4] "Uniform" Food, Drug and Cosmetic Law containing a special clause mentioning barbiturates and other drugs.
[5] State Narcotic Law as amended to include barbiturates and other drugs.
[6] "Uniform" Food, Drug and Cosmetic Law without a special clause mentioning barbiturates and other drugs.

## LEGAL REQUIREMENTS CONCERNING THE RETAIL SALE OF BARBITURATES AND OTHER HYPNOTIC DRUGS

All but one of the laws require that sales at retail must be made on prescription only. The exception is in the case of the law of New Hampshire, which is not a specific barbiturate law, but a general potent drug law which limits the sale of all drugs having an average adult dose of 60 gr. or minims or less to registered pharmacists or assistant pharmacists without requiring that all sales of such drugs must be made on prescriptions. Thirteen laws require that sales be made by pharmacists, two require that sales be made by registered drug stores only, one requires that sales be made by retail pharmacies only, and one specifies that the dealer must have a valid U. S. Narcotic license. A tabulation of the provisions of the laws is shown in Table III.

## RENEWAL OF PRESCRIPTIONS

Wide variation is found in the provisions of the laws and regulations with reference to the refilling of prescriptions. In only five laws is the refilling of prescriptions prohibited unconditionally. Fifteen laws make no reference to this subject and it is to be presumed that renewal is permitted. A tabulation of the provisions follows:

1. May not be refilled:
   Arkansas
   Colorado
   Louisiana[8]
   Michigan[7]
   Mississippi
2. All prescriptions except those for phenobarbital may not be refilled:
   Oregon
3. May be refilled on the written order of the prescriber:
   Nebraska
   Washington
4. May be refilled on the oral or written order of the prescriber:
   Connecticut
   Vermont
5. May not be refilled unless the prescriber endorses on the face of the prescription the specific number of times it may be renewed:
   Pennsylvania[8]
6. May not be refilled except on written or verbal order of the prescriber and then only provided the date of such renewal is recorded on the original prescription:
   Minnesota
7. May be refilled upon authorization of the prescriber:
   Alaska[9]

[7] Except prescriptions which are not designated otherwise by the prescriber may be refilled provided the barbituric acid and/or its derivatives are not the principal medicinal ingredients.
[8] By regulation.
[9] Refilling of prescriptions for barbiturates is not mentioned in the law. Authorization from the prescriber is required for renewal of prescriptions for chloral hydrate and sulfa drugs.

TABLE III.—LEGAL REQUIREMENTS CONCERNING THE RETAIL SALE OF BARBITURATES AND OTHER HYPNOTIC DRUGS

| Limited to Sales on Prescriptions | | May Be Sold by (Registered) Pharmacist Only | Sold by Registered Drug Stores Only | Sold by Retail Pharmacy Only | Dealer Must Have a Valid U. S. Narcotic License |
|---|---|---|---|---|---|
| Alabama | Nebraska[b] | Colorado[b] | Oklahoma | Arkansas | Mississippi |
| Alaska[a] | Nevada | Maryland | Rhode Island[d] | | |
| Arkansas | New Jersey[b] | Michigan | | | |
| California | New York | Minnesota | | | |
| Colorado[b] | North Carolina | Nebraska[b] | | | |
| Connecticut | Oklahoma | New Hampshire[c] | | | |
| Delaware | Oregon | Pennsylvania | | | |
| Florida | Pennsylvania | Puerto Rico | | | |
| Georgia[e] | Puerto Rico | New Jersey[b] | | | |
| Indiana | Rhode Island[d] | New York | | | |
| Louisiana | South Carolina | Rhode Island[d] | | | |
| Maine | Tennessee | Tennessee | | | |
| Maryland | Vermont | West Virginia | | | |
| Michigan | Virginia | | | | |
| Minnesota | Washington | | | | |
| Mississippi | West Virginia | | | | |
| Missouri | District of Columbia | | | | |

[a] Except that a licensed druggist may sell not more than 10 gr. of barbiturates without a prescription if the sale is recorded in the Poison Register.
[b] Must be compounded or dispensed by a registered pharmacist or under his immediate personal supervision.
[c] Except prescriptions containing not more than 4 grains of the specified drugs to the avoirdupois or fluid ounce.
[d] Prescriptions must be compounded or dispensed in a licensed pharmacy by a registered pharmacist or a registered assistant pharmacist, under the supervision of a registered pharmacist.
[e] Sale of all potent drugs is limited to registered pharmacists and assistant pharmacists. The only law that does not require prescriptions.

Virginia[10]

8. May be refilled but records must be kept:
   Delaware
   Florida[11]
9. All prescriptions except those containing less than two grains of phenobarbital per ounce and those containing diphenylhydantoin, aminopyrine and thyroid and derivatives may not be refilled:
   California
10. May be refilled two times provided authorization is obtained from the prescriber:
    District of Columbia[12]
11. May be refilled if prescriber does not state otherwise on the prescription:
    New Jersey[13]
    New York[14]
12. Renewal of prescriptions not mentioned in laws; (Presumably permitted):

| | |
|---|---|
| Albama | New Hampshire |
| Georgia | North Carolina |
| Indiana | Oklahoma |
| Maine | Puerto Rico |
| Maryland | Rhode Island |
| Missouri | South Carolina |
| Nevada | Tennessee |
| West Virginia | |

[10] Prescriptions containing derivatives of barbituric acid for persons suffering from chronic afflictions or diseases, and prescriptions for hormones may be refilled upon written authorization on the face of the prescription a definite number of times, as indicated, or indefinitely, if so specified. Prescriptions containing in a mixture or a compound a derivative of barbituric acid which does not constitute the principal constituent may be refilled (without permission) unless the prescriber indicates otherwise.
[11] Provided original is not designated by the prescriber as not to be refilled.
[12] By regulation.
[13] By regulation. Constant or frequent renewals, except for patients afflicted with epilepsy or other nervous diseases, are regarded as a "violation of the spirit of the law."
[14] No copies of prescriptions may be issued.

## REGULATION OF SALES BY MANUFACTURERS, JOBBERS, AND WHOLESALE DEALERS TO PHARMACISTS, PHYSICIANS, DENTISTS, VETERINARIANS, AND OTHER QUALIFIED PURCHASERS

Of the laws of the thirty-five states, district, or possessions now in force which regulate the distribution of barbiturates and/or other hypnotic drugs, only those of six states (Arkansas, California, Colorado, Michigan, Nebraska and New Jersey) contain, either in the law itself or in regulations supplementary thereto, any legal requirements affecting the sale at the wholesale level of these products. This relatively small number is explained in part by the fact that a majority of these laws are so worded as to limit their application to the "sale at retail or dispensing" of these drugs, with the result that wholesale transactions are not mentioned. In many other cases, all transactions except those at retail on prescription are prohibited, but a special clause is incorporated granting exemption to all legitimate sales at the wholesale level.

The legal requirements of the six laws which contain provisions regulating wholesale transactions are summarized below.

**Arkansas.**—All purchases of barbituric acid and derivatives and compounds thereof under any chemical or copyrighted name shall be ordered by the retail pharmacy or hospital or dispensing physician, dentist or veterinarian from the wholesale drug house, chemical house or manufacturer by written order, which order shall be made in triplicate, one copy to go to the wholesale drug house, chemical house or manufacturer, one copy to be sent to the

Food and Drug Division of the State Board of Health and the original or first copy to be retained by the pharmacy or dispensing physician, dentist or veterinarian placing the order. These orders shall be preserved by the wholesale drug house, chemical house or the manufacturer and by the pharmacy or hospital at least two years, subject to inspection of any officer of the State Board of Health.

**California.**—The provisions of the law do not apply to the sale of any dangerous drug by a manufacturer or wholesaler or pharmacy to each other or to a physician, dentist, chiropodist or veterinarian or to a laboratory under sales and purchase records that give currently the date, the names and addresses of the supplier and the buyer, the drug and the quantity. All records of manufacture and of sale or disposition of dangerous drugs shall be at all times, during business hours, open to inspection by authorized officers of the law, and shall be preserved at least three years.

**Colorado.**—Regulation 7 of the State Board of Pharmacy of Colorado provides the following: Sales of barbital and/or other hypnotic and somnifacient drugs by manufacturers to wholesalers, reretailers or medical practitioners; by wholesalers to retailers or to medical practitioners, and by one retailer to another, when carried out in good faith and not for the purpose of evading the provisions of this Act, are permitted without the use of a prescription. Invoices or bills of sale or equivalent records covering the sale of barbital and/or other hypnotic and somnifacient drugs under the conditions specified above must be kept on file by manufacturers, wholesalers, retailers and medical practitioners for a period of at least five years and must be open to inspection.

Sales of barbital and/or other hypnotic or somnifacient drugs by retailers to physicians, dentists and veterinarians must be made on prescription written by the medical practitioner and such prescriptions must be kept on file by the pharmacist in the same manner as other prescriptions under this Act.

**Michigan.**—"Manufacturers, wholesalers and retailers of drugs may sell, offer for sale, barter or otherwise dispose of, or be in possession of for sale, to licensed physicians, dentists, veterinarians, druggists, pharmacists, police laboratories and public health laboratories or hospitals, any of the following dangerous drugs: barbituric acid and any of its derivatives, chloral hydrate, or paraldehyde; provided, however, that a record of all such drugs, and their disposition, shall be kept, by the manufacturers, wholesalers or retailers, which record shall be open to inspection by an officer of any organized police force of this state or any prosecuting attorney or his investigators.

"All records required to be kept under the provisions of this Act shall be preserved for a period of two years."

**Nebraska.**—A pharmacist may dispense any of these drugs to a licensed physician, dentist or veterinarian who wishes to obtain them for dispensing to his patients, by taking a signed order for the same, which order shall be filed as if it were a prescription.

**New Jersey.**—Regulation 7 of the Board of Pharmacy of the State of New Jersey is identical in wording to that of Regulation 7 of the State Board of Pharmacy of Colorado, described above.

## DISPENSING OF BARBITURATES AND OTHER HYPNOTIC DRUGS BY PHYSICIANS, DENTISTS AND VETERINARIANS

The laws of only 16 states and one possession contain specific provisions authorizing the dispensing of barbiturates and other hypnotic drugs by licensed physicians, dentists, veterinarians or other medical practitioners. Seven of these laws contain provisions which require the keeping of records of all such transactions, or compliance with certain labeling requirements, or both.

No direct reference to dispensing by medical practitioners is found in the remaining 18 laws regulating the distribution of barbiturates and other hypnotic drugs. In many cases it is implied that dispensing by medical practitioners is permitted. However, two of these laws, those of Tennessee and West Virginia, contain provisions which, if interpreted literally, would have the effect of restricting all dispensing to that done by registered pharmacists.

The laws of the following contain provisions authorizing the dispensing of these drugs by licensed medical practitioners.

| | |
|---|---|
| Colorado | New Jersey[15] |
| Delaware[15] | New York[15] |
| Georgia[16] | Pennsylvania[15] |
| Maine | Puerto Rico[15,16] |
| Maryland | Rhode Island |
| Michigan | South Carolina[15] |
| Minnesota | Vermont[15] |
| Nebraska | Virginia |
| New Hampshire | |

The special provisions applying to dispensing by medical practitioners in the seven laws mentioned above, are summarized below.

**Delaware.**—An accurate record of the name and address of the patient, the date and quantity of the drug dispensed, as well as an accurate record of all renewals must be kept

**New Jersey and New York.**—A record must be kept of the date, the drug dispensed, the quantity, and the name and address of the patient.

**Pennsylvania and Puerto Rico.**—Records must be kept of the amount of such drugs purchased and a dispensing record must be maintained showing the date, name and quantity of the drug dispensed, and the name and address of the patient. The Pennsylvania law requires that the label shall show the name and address of the dispenser, the date dispensed, the name and address of the patient and

[15] Law contains provisions concerning the keeping of records or labeling requirements, or both.

[16] A physician is defined in the law as a person authorized to use, mix, prepare, dispense and administer drugs.

directions for use. The Puerto Rico law requires the label to show the name of the patient, the name of the physician and the date. The Pennsylvania law further provides that all purchasing and dispensing records shall be held for at least five years.

**South Carolina.**—The label must show the name and address of the prescriber (and the name and address of the dispenser).

**Vermont.**—Physicians, dentists and veterinarians are required to keep a record in a suitable book showing the names and addresses of all patients to whom these drugs are dispensed. The law provides that a veterinarian shall not prescribe any of the drugs mentioned in this act for the use of a human being.

As indicated above, it is questionable whether the dispensing by a medical practitioner in the states of Tennessee and West Virginia is permitted, except in the event that the practitioner also happens to be a registered pharmacist.

**Tennessee.**—The Tennessee law reads as follows:

"It shall be unlawful for any person, firm or corporation to sell, barter or give away any drug known as Barbital, as defined herein, except upon the written prescription of a duly licensed physician, dentist, or veterinarian, and compounded or dispensed by a duly registered pharmacist." No exemption clause is found elsewhere in the act authorizing the dispensing of these drugs by physicians, dentists or veterinarians.

**West Virginia.**—The pertinent provision of the West Virginia law reads as follows:

"Provided further, that in order to carry out the intent of this section to control the misuse of certain drugs that no Malonylurea or any of its salts, derivatives, mixtures or preparations thereof shall be sold or dispensed, at retail, by any person not a pharmacist as defined." Elsewhere in the law a pharmacist is defined to mean a licensed pharmacist. No provision is found elsewhere in the law permitting the dispensing of barbiturates by a physician, dentist or veterinarian.

## LABELING REQUIREMENTS APPLYING TO THE PHARMACIST

Analysis of the specific requirements of the existing laws concerning the information to be placed on prescription labels also shows a general lack of uniformity. Only 22 laws contain specific references to labeling requirements. However, the absence of a reference to a specific requirement does not mean necessarily that no such requirement exists in a given state, because there may be a general law imposing the requirement upon all prescription labels.

A summary of the information required on the label by specific provisions of laws is shown below.

1. Date:
   California, Colorado, Connecticut, Delaware, Florida, Georgia, Indiana, Maryland, Michigan, Missouri, Nebraska, Nevada, New Jersey, North Carolina, Pennsylvania, Puerto Rico, Washington
2. Prescription serial number:
   California, Colorado, Connecticut, Delaware, Florida, Indiana, Maryland, Missouri, Nebraska, Nevada, New Jersey, New York, North Carolina, Pennsylvania, Rhode Island, Washington
3. Name of pharmacist:
   Colorado, Delaware, Maryland, Nebraska, New Jersey, Pennsylvania, Rhode Island
4. Name of dispenser:
   Indiana, Michigan, Missouri
5. Name of the seller:
   Connecticut, Florida, Nevada, North Carolina
6. Name of the pharmacy:
   Washington
7. Name of the drug store or pharmacy:
   Georgia, Puerto Rico
8. Name of the supplier:
   California
9. Name of the owner of the establishment dispensing:
   New York
10. Name of the person, firm or corporation dispensing:
    South Carolina
11. Address of the pharmacist:
    Colorado, Delaware, Maryland, Nebraska, New Jersey, Pennsylvania
12. Address of the supplier:
    California
13. Place of business of the seller:
    Connecticut, Florida, Nevada, North Carolina
14. Address of the pharmacy:
    Washington
15. Address of the drug store or pharmacy:
    Georgia, Puerto Rico
16. Place of business of the dispenser:
    Indiana, Missouri
17. Address of the owner of the establishment dispensing:
    New York
18. Place of business of the pharmacist:
    Rhode Island
19. Address of the person, firm or corporation dispensing:
    South Carolina
20. Name of the prescriber:
    California, Missouri

Colorado, Connecticut, Delaware, Florida, Georgia, Indiana, Maryland, Nebraska, Nevada, New Jersey, New York, North Carolina, Pennsylvania, Puerto Rico, South Carolina

21. Address of the prescriber:
    South Carolina, Washington
22. Initials of the pharmacist compounding the prescription:
    Washington
23. Name of the person for whom prescribed:
    California
24. Name of the patient:
    Georgia, Michigan, Puerto Rico, Washington
25. Directions for use:
    California, Colorado, Delaware, Maryland, Minnesota, Nebraska, New York, Washington
26. "Use only as directed" label attached:
    Minnesota
27. "Cannot be renewed" label attached:
    District of Columbia

## EXEMPT PREPARATIONS

As shown in the discussion of legal requirements concerning the retail sale of barbiturates and other hypnotic drugs, 34 of the 35 laws reviewed require that sales at retail be on the prescription of a licensed medical practitioner. Thirteen of these laws make no provision for exemption from this requirement. The remaining 21 laws provide for so-called exempt preparations, but there is little agreement as to the types of preparations defined under this classification. The complete summary follows:

1. No exemptions:
   Alabama, Arkansas, California, Connecticut, Florida, Indiana, Maine, Michigan, Minnesota, Missouri, Nevada, North Carolina, Tennessee
2. Any preparation containing less than 1 gr. per avoirdupois or fluid ounce:
   Alaska
3. Preparations containing less than 1 gr. of barbiturates per avoirdupois or fluid ounce and preparations of sulfa drugs for external use and external veterinary use if approved as safe for such use by the State Board of Pharmacy:
   Washington
4. Preparations of chloral hydrate containing less than 2 gr. to the ounce:
   West Virginia
5. Preparations containing less than 4 gr. of the respective included drugs to the avoirdupois or fluid ounce:
   Georgia, Oregon, Puerto Rico
6. Preparations of benzedrine intended for nasal or other external use:
   Oklahoma
7. Preparations containing less than 10 gr. of barbiturates, derivatives of sulphonmethane, chloral, chloral hydrate and chlorobutanol per avoirdupois or fluid ounce and preparations of chloral, chloral hydrate and chlorobutanol when intended for external use:
   Delaware
8. Compounds containing not more than one-fourth the standard dose, provided other active medicinal ingredients present preclude the use of the compound to obtain the full effects of the barbiturate:
   South Carolina
9. Preparations containing not more than 3.0% of chlorobutanol present as an analgesic or analgesic and preservative in preparations containing one or more active ingredients and intended for parenteral use:
   Louisiana[17]
10. Preparations intended for external use:
    Vermont
11. Derivatives, compounds, preparations or mixtures of chloral, chloral hydrate or chlorobutonal intended for external use:
    Maryland
12. Preparations intended for external use and containing other agents conferring different medicinal properties:
    Colorado, New Jersey, New York, Pennsylvania, Rhode Island
13. Preparations containing drugs in the sulfanilamide group intended for external use and compounded so as to be unfit for internal use:
    Virginia
14. Compounds involving synergic action where the presence of other drugs render it incapable of being used for its hypnotic effects and compounds or mixtures or preparations used externally provided some other drug conferring different medicinal properties is present:
    Nebraska
15. Patent or common medicines handled by stores and commissaries for exclusive use in the treatment of sick mules, horses, cows, dogs, cats or other such animals:
    Mississippi
16. Acetanilid, in dosage of less than 5 gr. per day; acetophenotidin or antipyrine, in dosage of less than 15 gr. per day; benzedrine sulfate, for external use; bromides, in dosage less than 30 gr. per day or less than 15 gr. in any three-hour period; bromide-acetanilid, in dosages less than 15 gr. of bromide per day and 5 gr. of acetanilid per day or less than $7\frac{1}{2}$ gr. of bromide and less than $2\frac{1}{2}$ gr. of acetanilid in any three-hour period; cantharides, for external use; ephedrine solution, less than 1%; ipecac, in dosages less than 10 gr. per day or when given in larger dosage as a prompt emetic; phenol, for external use, if less than 2% or if directions for use indicate dilution to less than 2%; squill, in dosages of less than 5 minims of tincture and $7\frac{1}{2}$ minims of syrup and strychnine and all of its salts in dosages of less than $\frac{1}{20}$ gr. per day:
    District of Columbia[17]

[17] By regulation.

## ILLEGAL POSSESSION

The laws of seven states and one possession contain clauses which state that possession, by persons other than manufacturers, wholesalers, pharmacists, medical pratitioners, laboratories, hospitals and other authorized individuals, is unlawful, except under certain conditions. These laws are those of Arkansas, California, Georgia, Michigan, Minnesota, Mississippi, Puerto Rico and South Carolina. The conditions under which possession becomes a violation of the law are summarized below:

1. Possession other than as authorized in the respective laws:
   Arkansas
   Minnesota
2. If the drug is not in the original container in which it was dispensed by the pharmacist or physician:
   Georgia
   Michigan
   Puerto Rico
3. Unless the label shows the name and address of the prescriber and the name and address of the dispenser;
   South Carolina
4. Unless furnished on the prescription of a physician, dentist, chiropodist or veterinarian:
   California
5. Unless prescribed by a physician, dentist or veterinarian possessing a valid U. S. Narcotic License:
   Mississippi

The laws of six other states—Colorado, Delaware, Maryland, Nebraska, New Jersey, Pennsylvania and Rhode Island—all contain clauses making it unlawful for a manufacturer, pharmacist or other dealer to hold these drugs in his possession unless the container has a label attached stating in printed words the specific name of the drug and the proportion or amount thereof. This provision does not apply to a drug dispensed on a prescription.

## VIOLATIONS

The law of Minnesota describes a violation as a gross misdemeanor; the laws of 22 states[18], the District of Columbia and Puerto Rico designate the violation as a misdemeanor. The violation is not classified in the laws of the remaining nine states[19] and Alaska, although specific penalties are established in the laws of seven of these.[20] In addition, the laws of Georgia and Alabama, although defining the violation as a misdemeanor, do not state the penalty specifically.

[18] Alabama, Arkansas, California, Colorado, Florida, Georgia, Indiana, Louisiana, Maryland, Michigan, Mississippi, Missouri, Nebraska, Nevada, New York, North Carolina, Oklahoma, Oregon, Pennsylvania, South Carolina, Tennessee, Virginia.

[19] Connecticut, Delaware, Maine, New Hampshire, New Jersey, Rhode Island, Vermont, Washington and West Virginia.

[20] Alaska, Connecticut, Maine, New Hampshire, New Jersey, Vermont, West Virginia.

## PENALTIES

The laws of five states—Delaware, Georgia, Mississippi, Rhode Island and Washington—fail to state, in the law covering barbiturates, the exact penalty for violation. Two of these laws, those of Georgia and Mississippi, classify the violation as a misdemeanor and provide that the punishment should be that provided elsewhere by law for such an offense.

The laws of seven states establish fines as the only penalty. Five of these laws make no distinction between first and subsequent offenses. The laws of two of these states, New Jersey and Pennsylvania, provide for increased fines for second, and for third and subsequent offenses.

The laws of ten states and Alaska and Puerto Rico assign penalties involving fines or imprisonment or both, and with one exception (California) make no distinction between first and subsequent offenses. However, the California law specifies that upon a third conviction proceedings shall be initiated for the forfeiture of the professional license. This is the only law which makes any reference to the revocation of licenses as a possible penalty. The law of Puerto Rico provides for imprisonment up to ninety days, or a fine of $1.00 per day for each day of the sentence that is not served.

Nine states and the District of Columbia provide for penalties involving fines or imprisonment, or both, and establish increased penalties for second and subsequent violations.

The laws of two states, Nebraska and Colorado provide for penalties involving fines and imprisonment, or both, and establish a series of penalties on an increasing scale for first, for second and for third and subsequent offenses. The Nebraska law does not impose imprisonment as a part of the penalty except for third and subsequent offenses.

A tabulation of the penalty provisions of the thirty laws containing them is shown in Table IV.

## SUMMARY

As of October 1, 1945, thirty-two states, Alaska, Puerto Rico and the District of Columbia had laws in force which either directly or indirectly control, or could be used for the purpose of controlling the distribution of barbiturates or barbiturates and other drugs. Four additional states exercise control by regulation by appropriate agencies of the state governments in the absence of statutes. Six states having laws and the District of Columbia have promulgated regulations dealing with the control of these drugs.

The first state law on barbiturates was that of California, enacted in 1929. This

TABLE IV.—PENALTIES

I. Penalties Involving Fines Only

A. Single Offenses

| Fine | State |
|---|---|
| $10.00 to $100.00 | Arkansas |
| Up to $100.00 | Maryland |
| $10.00 to $250.00 | Oklahoma |
| $10.00 to $500.00 | Alabama |
| $25.00 to $250.00 | Tennessee |

B. Including First, Second and Third, and Subsequent Offenses

First offense, $25.00 to $50.00; second offense, $50.00 to $100.00; third and subsequent offenses, not less than $100.00—New Jersey, Pennsylvania

II. Penalties Involving Fines or Imprisonment, or Both

A. Single Offenses

| Fine | Imprisonment | State |
|---|---|---|
| $50.00 to $500.00 | Up to 6 mo., or both | California[a] |
| $50.00 to $500.00 | Up to 1 yr., or both | New Hampshire |
| $50.00 to $1000.00 | Up to 1 yr., or both | Vermont |
| $100.00 to $500.00 | Up to 180 days, or both | Alaska |
| Up to $100.00 | Up to 6 mo., or both | Maine |
| Up to $200.00 | Up to 6 mo., or both | Oregon |
| Up to $500.00 | Up to 6 mo., or both | Nevada |
| Up to $500.00 | Up to 1 yr., or both | Michigan |
| Up to $500.00 | Up to 18 mo., or both | South Carolina |
| | Up to 90 days | Puerto Rico[b] |
| Up to $100.00 | Up to 1 yr., or both | Minnesota, Missouri |

B. First and Second and Subsequent Offenses

| | First Offense | Second and Subsequent Offenses | State |
|---|---|---|---|
| Imprisonment | ..... | Up to 6 mo. | Virginia |
| Fine | Up to $100.00 | Up to $1000.00, or both | |
| Imprisonment | Up to 6 mo. | Up to 1 yr. | Florida |
| Fine | Up to $500.00, or both | Up to $1000.00, or both | Connecticut |
| Imprisonment | Up to 6 mo. | Up to 12 mo. | North Carolina |
| Fine | Up to $200.00, or both | Up to $400.00, or both | |
| Imprisonment | Up to 6 mo. | Up to 2 yr. | Indiana |
| Fine | $10.00 to $1000.00, or both | $50.00 to $2000.00, or both | |
| Imprisonment | Up to 1 yr. | Up to 2 yr. | Louisiana |
| Fine | Up to $1000.00, or both | Up to $3000.00, or both | |
| Imprisonment | Up to 1 yr. | Up to 2 yr. | New York |
| Fine | Up to $500.00, or both | Up to $1000.00, or both | |
| Imprisonment | Up to 1 yr., or both | Up to 5 yr., or both | |
| Fine | Up to $100.00 | Up to $1000.00 | West Virginia |
| Imprisonment | Up to 1 yr. | Up to 3 yr. | District of Columbia[c] |
| Fine | Up to $1000.00, or both | Up to $10,000.00, or both | |

C. First, Second and Third, and Subsequent Offenses

| | First Offense | Second Offense | Third and Subsequent Offenses | State |
|---|---|---|---|---|
| Imprisonment | ..... | ..... | One year, or both | |
| Fine | $25.00 to $50.00 | $50.00 to $100.00 | Not less than $100.00 | Nebraska |
| Imprisonment | Up to 6 mo. | Up to 6 mo. | Up to 6 mo. | |
| Fine | $25.00 to $50.00 | $50.00 to $100.00 | Not less than $200.00 | Colorado |

[a] Proceedings for forfeiture of professional license shall be initiated for the third and subsequent offenses.
[b] Or a fine of $1.00 per day for each day of the sentence which is not served.
[c] Except that a first conviction with intent to defraud or mislead shall be punished as if for a second or subsequent offense.

law has since been repealed and replaced by a new law adopted in 1945. Oklahoma replaced its former law of 1933 by a new law in 1945 and New York adopted a new law in 1945.

The existing laws may be classified as special laws, covering barbiturates, either including or excluding other hypnotics and/or other drugs, and general laws such as the so-called Uniform Food, Drug and Cosmetic Laws or the State Narcotic Laws. Twenty-five of the laws are special laws and ten are general laws.

Wide differences are noted in the drugs covered by these laws and regulations, varying from barbiturates only, as is the case in the laws of Arkansas, Maine, Oregon, South Carolina and Tennessee, to the broad

coverage of the New Hampshire law, which includes all drugs having a dose of 60 gr. or minims or less. In some of the general laws, barbiturates and, in some cases, other hypnotic agents are incorporated in the narcotic laws. In others where the State Uniform Food, Drug and Cosmetic Act is used as the legal basis for control, the tendency is to include the barbiturates with dangerous drugs, such as derivatives of sulfanilamide, cinchophen, dinitrophenol and similar drugs.

All but one of the 35 laws, that of New Hampshire, restrict the sale of barbiturates, or barbiturates and other drugs, to prescriptions. In this case the general law on potent drugs limits the sale of all such drugs to a registered pharmacist or assistant pharmacist. Thirteen laws require that sales must be made by pharmacists, two require that sales be made by registered drug stores only, one requires that sales be made by retail pharmacists only and one specifies that the dealer must have a valid U. S. Narcotic license.

Five laws prohibit the renewal of prescriptions; one permits the renewal of prescriptions for phenobarbital only; two permit refills upon the written order of the prescriber only; two permit renewals with either the oral or written order of the prescriber; another permits a refill under the same conditions provided the date of such renewal is recorded on the original prescription; refills are permitted in another law only if the prescriber endorses on the face of the prescription the specific number of times the prescription may be refilled; two laws merely require that authorization be obtained from the prescriber; two laws require that records be kept of all refills; one state permits the refilling of only those prescriptions calling for less than 2 gr. of phenobarbital per ounce and those containing diphenylhydantoin, aminopyrine and thyroid and derivatives; two state laws permit refills if the prescriber does not state otherwise on the prescription and the regulations in effect in the District of Columbia limit the number of refills to two. Fifteen laws make no reference to the refilling of prescriptions.

Only six laws make any provisions for controlling sales at the wholesale level. One provides for triplicate order blanks, one copy to be sent to the supplier, another to be filed with the Food and Drug Division of the State Board of Health, and the third to be retained. The regulations of three states provide for the preservation of all invoices, bills-of-sale or equivalent records, subject to inspection. One law requires records to be kept of the manufacture, sale and disposition of barbituric acid and its derivatives, chloral hydrate and paraldehyde. Another law states that a pharmacist may dispense the included drugs to a licensed physician, dentist or veterinarian upon a signed order which must be filed as if it were a prescription.

Only 17 laws specifically authorize the dispensing of barbiturates by licensed medical practitioners, and seven of these laws impose requirements either for labeling or for the keeping of records, or both. The laws of two states apparently limit all dispensing of these drugs to registered pharmacists.

An analysis of the labeling requirements which must be observed by the pharmacist shows a marked lack of uniformity in these requirements. Only 22 laws make specific reference to this subject. However, it is probable that in many cases detailed requirements are stated elsewhere in general laws which were not included in this survey.

Thirteen of the 34 laws limiting retail sales to prescriptions make no provision for exemption from this requirement. The remaining 21 laws recognize certain preparations as exempt, but the exemptions are identical only in the case of three of these laws. This wide disparity is partially due to the differences in the drugs covered by the individual laws.

Illegal possession is made a violation of the laws of seven states and Puerto Rico. The conditions under which possession is declared illegal are: possession other than as authorized by law; if not in the original container in which it was dispensed by the pharmacist or physician; unless the label shows the name and address of the prescriber and the name and address of the dispenser; unless furnished on the prescription of a

physician, dentist, chiropodist or veterinarian; unless prescribed by a practitioner possessing a valid U. S. Narcotic license.

Violations are defined as gross misdemeanors by one state, as misdemeanors by 22 states, the District of Columbia and Puerto Rico, and not specified by nine states and Alaska.

Penalties for violations cover a wide range and have been specifically stated by twenty-seven states, Alaska, Puerto Rico and the District of Columbia. Fines extend from a minimum of ten dollars to a maximum of ten thousand dollars and prison terms vary from a minimum of ninety days to a maximum of five years.

# The Alkaloids of Species of Garrya

## I. Isolation of Alkaloids*

By J. F. ONETO

**Six species of Garrya have been examined for their alkaloidal content. *G. flavescens* contained no alkaloids while *G. fremontii* and *G. buxifolia* yielded amorphous, basic, nitrogenous residues which did not form crystalline salts. *G. elliptica, G. veatchii,* and *G. wrightii,* on the other hand, all contained alkaloids which were isolated as crystalline hydrochlorides. Two crystalline substances were isolated from the bark of *G. veatchii* and a number of their salts have been prepared. The proposed names and provisional empirical formulas for these two substances are (*a*) Garryine, $[C_{22}H_{32}O_2N(H_2O)]$ and (*b*) Veatchine, $(C_{22}H_{32}O_2N)$.**

THIS investigation was inaugurated in the search for quinine substitutes on the basis that reports of the use of Garrya in the treatment of intermittent fevers have appeared in the literature (1).

According to Baily (2), Garrya is now considered to be the only genus in the monotypic family *Garryaceae*. At one time, however, this genus, named for Nicholas Garry, secretary of the Hudson Bay Company, was included in the dogwood family, *Cornaceae*.

The plant materials from the various Garryas, suitably ground and dried for extraction, were identified by and supplied through the United States Department of Agriculture, Forest Service, California Forest and Range Experimental Station, Berkeley, California.[1]

Because of the desirability of obtaining alkaloidal residues as rapidly as possible in sufficient quantities for antimalarial evaluation,[2] exhaustive phytochemical studies of the available Garrya species were not undertaken.

In an early publication Ross (3) described the isolation of a few cubical crystals of a very bitter substance from two ounces (Troy) of the dry leaves of *G. fremontii*. This substance, isolated in the form of the hydrochloride, was described briefly as being soluble in water and alcohol and as giving a positive test with Mayer's reagent. When treated with concentrated sulfuric acid it developed a purple color. Ross named this substance "Garryina." It is significant to note that the samples of *G. fremontii* upon which we conducted part of our investigations yielded a noncrystalline nitrogenous base which did not form crystalline salts or produce a purple color when treated with concentrated sulfuric acid. In a later publi-

* Received March 13, 1946 from the University of California, College of Pharmacy, San Francisco.

[1] We wish to acknowledge the splendid cooperation of members of the California Forest and Range Experimental Station during the progress of this investigation.

[2] The antimalarial evaluations of certain extracts and alkaloidal residues prepared in this laboratory from various species of Garrya were determined by The National Research Council, Survey of Antimalarial Drugs.

cation Moeller (4) mentioned that the leaves of *G. fremontii* contain bitter, water-soluble substances which were isolated as a crumbly mass. The method of isolation was not described. Armendariz (5) isolated alkaloidal material from the bark of *G. racemosa*, Ramirez. The material was described as being very bitter and readily soluble in water and alcohol. As in the case of the substances isolated by both Ross and Moeller, that which was isolated by Armendariz was insufficiently characterized for comparison.

## EXPERIMENTAL

**General Methods for the Isolation of Alkaloidal Residues as the Hydrochloride Salts.**—Fluidextracts of 500-Gm. quantities of the ground plant materials were prepared according to the U. S. P. XII, Type Process A. The menstruum employed consisted of 19 volumes of 95% alcohol diluted with 5 volumes of water. In each instance, the fluidextracts thus prepared were concentrated to a thick syrupy residue by reduced pressure distillation on the steam bath. The residue was then treated with 300–400 cc. of water and allowed to stand overnight. The mixture was subsequently filtered with suction, the precipitate washed repeatedly with water and the washings added to the original filtrate. The combined filtrates were concentrated on the steam bath to a convenient working volume, made alkaline with 25 cc. of 40% sodium hydroxide solution and completely extracted with chloroform. The chloroformic extract was in turn extracted with 3% sulfuric acid, the acid extracts rendered strongly alkaline with 40% sodium hydroxide solution and again extracted with chloroform. The latter steps of the extraction procedure were repeated until the chloroformic extract was practically colorless. The completeness of extraction during the various steps was continually checked by the use of Mayer's reagent. The final chloroformic extract was concentrated by distillation on the steam bath to a small volume, transferred to a desiccator, and the remaining chloroform removed under reduced pressure. The resulting dry alkaloidal residue was dissolved in absolute alcohol, refluxed with decolorizing carbon, and filtered. The filtrate was concentrated to a small volume on the steam bath and then acidified with a solution of 37% hydrochloric acid in absolute alcohol. Concentration to dryness was effected in an evacuated desiccator over calcium chloride and sodium hydroxide. (An alternate method was to treat the concentrated acid alcoholic solution with an excess of ether whereupon the alkaloidal material precipitated as the crystalline hydrochloride.) The dry residue was purified by dissolving in warm absolute alcohol and adding an excess of warm ether to the alcoholic solution. The alkaloidal material precipitated as the hydrochloride in the form of colorless crystals on standing.

Of the six species of Garrya which were investigated, *G. flavescens* contained no alkaloidal material while *G. fremontii* and *G. buxifolia* yielded amorphous, basic, nitrogenous residues which responded positively to a number of the general alkaloidal reagents. The residue from the two latter Garryas appeared to undergo progressive decomposition during attempts at purification and salt formation. Application of the lead acetate method to extracts of *G. fremontii* gave rise to a glycosidic material which was not definitely characterized but which responded to a number of general tests for glycosides. Residues from the three remaining Garryas all formed crystalline hydrochlorides.

TABLE I.—SUMMARY OF EXTRACTION STUDIES OF SIX DIFFERENT SPECIES OF GARRYA

| Species | Part Used | Collection Area | Collection Date | Yield of Alkaloidal Hydrochloride, Gm./500 Gm. | M. P. (Uncor.), °C. |
|---|---|---|---|---|---|
| *G. buxifolia* | Leaves | Humboldt County, Calif. | 4/43 | [a] | ..... |
| *G. elliptica* | Leaves | Marin County, Calif. | 10/42 | 0.22 | 250–251 (dec.) |
| *G. flavescens* | Leaves | Santa Barbara Co., Calif. | 6/43 | [b] | ..... |
| *G. fremontii* | Leaves | Tuolumme County, Calif. | 6/43 | [a] | ..... |
| *G. veatchii* | Leaves | Los Angeles County, Calif. | 5/42 | 1.85 | 252–253 (dec.) |
| *G. veatchii* | Bark | Los Angeles County, Calif. | 5/42 | 3.00 | 251–252 (dec.) |
| *G. wrightii* | Leaves | Donna Ana County, N. Mexico | 5/42 | 1.00 | 251–252 (dec.) |
| *G. wrightii* | Bark | Donna Ana County, N. Mexico | 5/42 | 0.95 | 250–251 (dec.) |

[a] Yields of impure, amorphous bases not recorded.
[b] *G. flavescens* gave no evidence of containing alkaloidal material.

The results of the application of the above general method for the isolation of alkaloidal residues to the various Garrya species are shown in Table I.

**Large-Scale Extraction of G. veatchii Bark.**—Approximately 3.5 Kg. of ground *G. veatchii* bark was extracted as described in the previous method. On evaporation of the final chloroformic extract 27.5 Gm. of crude, crystalline alkaloidal base was obtained. This residue was light yellowish brown in color, possessed a slight amine-like odor and melted at approximately 110–118°.[3] The entire yield was purified by converting to the hydrochloride in the usual way with subsequent recrystallization using alcohol and ether. The yield of crystalline hydrochloride after several crystallizations was 22 Gm; m. p. 251–252° (dec.). An additional 6.5

[3] All melting points are uncorrected.

Gm. of the crystalline hydrochloride was obtained upon working up the mother liquors from the first series of recrystallizations; m. p. 251–252° (dec.).

**Fractional Separation and Purification of the Alkaloids of G. veatchii Bark**

**Fraction A.**—Ten grams of the purified crystalline hydrochloride obtained from the extraction of *G. veatchii* bark was dissolved in 200 cc. of water. The *p*H of the aqueous solution was 4.15. (*p*H determinations were made with a Coleman *p*H Meter, No. 200, using a glass electrode.) Employing a 25% aqueous solution of potassium hydroxide, the *p*H of the alkaloidal solution was adjusted to 7.20. The copious precipitate of free base which formed was removed by suction filtration, washed with a little cold water and dried over calcium chloride; yield 2.28 Gm. After several recrystallizations from 50% alcohol the substance did not give a satisfactory melting point indicating possibly a mixture, or solvent of crystallization or both. When heated in a melting-point apparatus it began shrinking at about 88°, and at approximately 96° appeared as an opalescent vitreous blob on the side of the melting point tube.

The recrystallized fraction A, as well as subsequent purified fractions obtained from the separation, was prepared for analysis by desiccating over calcium chloride at reduced pressure for a period of three days at room temperature. No change in their behavior toward heating was noted for any of the various fractions after this treatment.

*Anal.*[4]—Calcd. for $C_{22}H_{34}O_3N$: C, 73.29; H, 9.50; N, 3.88. Found: C, 73.34 (av.); H, 9.49 (av.); N, 3.96 (av.).

Solvent of crystallization:—Calcd. for $C_{22}H_{32}O_2N$-($H_2O$): $H_2O$, 4.99. Found: 4.65.

This result was based on the loss in weight of 6.1 mg. when 0.1310 Gm. of purified fraction A was dried to constant weight in an evacuated Abderhalden apparatus over phosphorus pentoxide. After heating for three and one-half hours using 95% alcohol as the reflux liquid the base had fused to an opalescent mass. An additional heating for a two-hour period using water as the reflux liquid converted the opalescent mass to a clear vitreous varnish. The dry, glassy material did not give a sharp melting point, but when heated, melted sluggishly over a long range, starting about 67°.

**Derivatives.**—A general method utilized for the rapid and expedient preparation of colorless salts of inorganic acids of the various alkaloidal fractions isolated in the fractional studies is described as follows: Fifteen to 25 mg. of the purified alkaloidal fraction was dissolved in a small amount of warm, absolute alcohol. The warm alcoholic solution was then rendered acid with a freshly prepared dilute solution of the corresponding inorganic acid in absolute alcohol. Upon treating the warm acid solution with dry, warm peroxide-free ether until a heavy cloudiness resulted, the corresponding alkaloidal salt invariably precipitated in crystalline form on standing. The crystalline material was removed by suction filtration and immediately recrystallized by dissolving in warm alcohol and again adding warm peroxide-free ether. The recrystallized material was dried in a desiccator under vacuum.

Crystalline Salts of Fraction A:
Hydrochloride, m. p. 251–252° (dec.).
Hydrobromide, m. p. 229–230° (dec.).
Hydroiodide, m. p. 203–204° (dec.).
Sulfate, decomposes gradually over a long range on being heated in a melting-point apparatus.

The mother liquors obtained from the recrystallizations of fraction A were combined and concentrated to a small volume on the steam bath. The dealcoholized concentrate was extracted with chloroform after the addition of 5 cc. of 40% sodium hydroxide solution. The chloroformic extract was evaporated to dryness and the residue recrystallized from 50% alcohol; yield 0.27 Gm. The melting point of the corresponding hydrobromide was 229–230° (dec.); mixed m. p. 229–230° (dec.).

The filtrate from the 0.27 Gm. of recrystallized material was concentrated to a small volume and allowed to crystallize; yield 75 mg. Converted to the hydrobromide it melted at 226–227° (dec.) without being recrystallized.

**Fraction B.**—The alkaline filtrate from the original precipitation of fraction A (*p*H 7.20) was adjusted with 25% aqueous potassium hydroxide to *p*H 9.30. At this point the addition of small portions of the potassium hydroxide solution failed to cause further precipitation. The colorless curdy precipitate was removed by filtration, washed with small portions of cold water and dried; yield 1.66 Gm. Recrystallized from 50% alcohol the material behaved as did fraction A when heated in a melting-point apparatus.

*Anal.*—Calcd. for $C_{22}H_{34}O_3N$: C, 73.29; H, 9.50; N, 3.88. Found: C, 73.45 (av.); H, 9.33 (av.); N, 3.89 (av.).

Mixed melting-point observations of the hydroiodide salts prepared from fractions A and B showed no melting-point depression. From the various analyses and other melting-point data it was concluded that fractions A and B, recrystallized from 50% alcohol, are identical substances.

The specific rotation of the purified crystalline material in a concentration of 1.4364 Gm. per 100 cc. of solution in alcohol was $[\alpha]_D^{27.5} = -84.23°$.

It is suggested that the name Garryine, first proposed by Ross (3), be retained for this substance, for convenience of reference.

The equivalent weight of Garryine was determined by dissolving 41.6 mg. of the base in 70 cc. of 50% neutral alcohol with the aid of heat, cooling to room temperature and titrating with 0.01 *N* sulfuric acid to a methyl red end point. The titration re-

[4] Analyses by Mr. C. Koch, Chemistry Department, University of California, Berkeley, Calif.

quired 11.858 cc. of the standard acid, indicating an equivalent weight of 350.8; calculated, 360.4.

**Fraction C.**—The alkaline filtrate obtained from the precipitation of fraction B (*p*H 9.3) was treated with sufficient 25% aqueous potassium hydroxide to produce a permanent, colorless precipitate. The precipitate was filtered off and dried in the usual way; yield 1.76 Gm.; m. p. 120–123°. The free base was purified by dissolving in acetone, refluxing with decolorizing carbon, and filtering. The filtrate was concentrated to a small volume and then the warm, concentrated acetone solution was treated with water until a distinct cloudiness resulted. The supernatant liquid became clear as crystallization proceeded. Additional small portions of water were added from time to time until crystallization was complete. After several recrystallizations in this manner the melting point of fraction C was 122–123°.

*Anal.*—Calcd. for $C_{22}H_{32}O_2N$: C, 77.12; H, 9.41; N, 4.08. Found: C, 76.89 (av.); H, 9.37 (av.); N, 4.12 (av.).

Derivatives of Fraction C:
Hydrochloride, m. p. 251–252° (dec.).
Hydrobromide, m. p. 229–230° (dec.).
Hydroiodide, m. p. 222–223° (dec.).

On working up the mother liquors obtained from the purification of fraction C there was obtained about 0.20 Gm. of crystalline material melting at 93–115°, with preliminary shrinking. This material was fractionated at different *p*H concentrations to yield (*a*) one fraction, which when converted to the hydroiodide melted at 201–202° without being recrystallized, and (*b*) a second fraction which was converted to the hydrobromide giving a melting point of 229–230° (dec.).

**Fraction D.**—The alkaline filtrate from the precipitation of fraction C was completely extracted with chloroform after the addition of 40 cc. of 25% aqueous potassium hydroxide. The chloroformic extract was reduced to a small volume by distillation on the steam bath, then allowed to evaporate spontaneously to a crystalline residue. The yield was 3.35 Gm., m. p. 120–123°. The base was purified by the acetone-water method; m. p. 122–123°. This material showed no loss in weight when heated in an evacuated Abderhalden apparatus over phosphorus pentoxide for a period of several hours using water as the reflux liquid.

*Anal.*—Calcd. for $C_{22}H_{32}O_2N$: C, 77.12; H, 9.41; N, 4.08. Found: C, 77.13 (av.); H, 9.41 (av.); N, 4.11 (av.).

The crude crystalline material recovered from the mother liquors melted at 114–117°, with preliminary shrinking. This material was not investigated further.

The various analyses, as well as melting point data of derivatives, indicate that fractions C and D are identical substances.

The specific rotation of the crystalline substance in a concentration of 1.0578 Gm. per 100 cc. of solution in alcohol was $[\alpha]_D^{27.5} = -69.01°$.

The equivalent weight of fraction D was determined in precisely the same manner as employed for fraction B. Sixty and nine-tenths milligrams of the free base required 17.34 cc. of 0.01 *N* acid for neutralization to methyl red indicator. Equivalent weight found, 351.2; calculated, 342.4.

It is proposed that this substance be named Veatchine, after the species of Garrya from which it was isolated, for convenience of reference.

When a few crystals of either Garryine or Veatchine were treated with concentrated sulfuric acid on a spot plate an orange color developed which slowly changed to a deep red and finally assumed a deep purple color on long standing.

## SUMMARY

1. Amorphous nitrogenous bases have been isolated from *G. fremontii* and *G. buxifolia*, none of which formed crystalline hydrochlorides. *G. elliptica*, *G. veatchii*, and *G. wrightii*, on the other hand, gave rise to crystalline nitrogenous bases which formed crystalline hydrochlorides. *G. flavescens* contained no alkaloidal material.

2. Two crystalline substances have been isolated from the bark of *G. veatchii* and a number of their salts have been prepared. It is proposed that these substances be named Garryine and Veatchine, for convenience of reference.

3. Garryine does not give a sharp melting point but forms a hydroiodide melting at 202–203° (dec.). The provisional empirical formula for Garryine based on a minimal nitrogen content of one nitrogen atom is $C_{22}H_{32}O_2N(H_2O)$.

4. Veatchine melts at 122–123° and forms a hydroiodide melting at 222–223° (dec.). The provisional empirical formula for Veatchine based on the same assumption as above is $C_{22}H_{32}O_2N$.

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# Sweat- and Water-Resistant Sunburn Preparations*,†

By ARTHUR C. GIESE and JULIAN M. WELLS‡

**No preparation having the properties of an ideal sunburn preventive is available and none has been produced, but an approach to it has been made. Of some 100 formulations tried, a bentonite clay ointment, a stearate mixture base ointment, a vanishing cream, and an ethocel lotion, nearly all containing homomenthyl salicylate and in some cases also ethyl *p*-aminobenzoate as sunscreens and titanium dioxide as the pigment proved most satisfactory. The value of the ointments in sunburn protection was tested by comparing the ratio of the dosage required in the control patch of skin. Sweating and washing with water decrease the protective value of the ointments but not as much as in the case of commercial ointments tried. The formulas for the new preparations are given in full.**

MOST COMMERCIAL sunburn ointments and lotions are easily washed or wiped off. From some standpoints this is highly desirable, since under ordinary sunbathing conditions the bather hesitates to apply anything to the skin which is not readily removed. For some purposes, where long and continuous exposure to the sun in humid climates is necessary and where it is not feasible to reapply the ointment periodically, a more permanent coating is desirable. In the present report several ointments are described which have withstood tests of sweating and abrasion satisfactorily.

The ideal sunburn preparation should satisfy the following requirements: It should (*a*) be easy to apply uniformly over smooth and hairy surfaces; (*b*) dry rapidly, leaving a film with no sticky or greasy feel; (*c*) resist the action of moisture but permit sweat and heat transfer; (*d*) resist mechanical rubbing; (*e*) wash off with soap and water; (*f*) remain stable under varying temperature conditions and after subjection to sunlight; (*g*) be nonirritating and nonsensitizing; (*h*) require only readily obtainable materials; (*i*) provide effective protection in relatively thin films.

No ideal preparation has yet appeared, but some fairly satisfactory ones have been made. Such sunburn preparations are generally mixtures of a number of compounds and contain: (*a*) a base or vehicle in which all the ingredients are incorporated; (*b*) a sunscreen, which selectively absorbs the sunburn radiations in sunlight; (*c*) a scattering or light-dispersing agent, such as titanium dioxide; (*d*) pigments to match the color of the skin; and (*e*) miscellaneous substances which serve either to modify the properties of other materials or to keep the ointment from spoiling. Since the sweat and abrasion resistance of the preparation depends mainly on the type of base or vehicle used, this portion of the report is chiefly concerned with different types of vehicles. However, the vehicle must be built about the sunscreen and a brief consideration of sunscreens is consequently desirable.

Many sunscreens have been used and a fairly detailed list may be found in de Navarre's book (1); still other compounds are listed in *Chemical Abstracts* from about 1930 to the present, the greatest number appearing just before the war. Most of these compounds fall into a few "families"; namely, the *p*-aminobenzoates, anthranilates (*o*-aminobenzoates), salicylates (*o*-hydroxybenzoates), cinnamates, pyrrones (e.g., aesculin), benzimidazoles, carbazoles, and naphthol sulfonates. However, a number of miscellaneous chemicals have also been employed. Only a few of the compounds have been used extensively and their behavior in ointments still remains to be studied since some are unstable to ultraviolet light, some irritate the skin, others have undesirable odors, etc.

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† The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Stanford University and the University of California.

‡ We are indebted to Messrs. George Fohlen, Francis Haxo, and Howard Nicholson for aid in carrying out some of the experiments.

In the present study it was necessary to choose arbitrarily a few of the possible sunscreens, to test these in a preliminary fashion and then to build the preparation about those which appear to have the most satisfactory properties. Preliminary testing indicated that homomenthyl salicylate and ethyl *p*-aminobenzoate had satisfactory properties. Our studies were therefore confined to them but undoubtedly other sunscreens with similar properties could be substituted and the studies described below will serve to illustrate the methods and problems involved in developing a preparation with sweat and abrasion resistance.

## EXPERIMENTAL

**Experiments on Vehicles.**—The conventional bases used as vehicles for sunscreens may be classified as:

(*a*) Lotions, *1*, hydroalcoholic and *2*, oily.
(*b*) Vanishing creams (o/w emulsions).
(*c*) Fatty or greasy bases, usually anhydrous in character.

The bases in use do not have the sweat resistance or abrasion resistance desired; therefore, an attempt was made (a) to modify them in such a manner as to develop or improve the qualities desired and (b) to develop new bases which might show these properties. Over 100 combinations were prepared and studied. The bases other than the conventional ones listed above which were tried are: bentonite, methyl cellulose, sodium alginate (Kelgin), pectin, keratin, resins, gum resins, balsams, polyvinyl alcohol, vinyl resin, ethyl cellulose, and tincture of benzoin. We shall first discuss the new bases and, second, the modifications of the conventional bases. The sweat, water, and abrasion resistance of the preparations will be discussed in the following section.

Bentonite, a colloidal native hydrated aluminum silicate, was selected for experimental work. It forms a stable gel with water and is compatible with many chemicals. The gel spreads easily on smooth or hairy skin surfaces, dries rapidly leaving a uniform film that is not sticky or too easily rubbed off. However, the film is not resistant to applied water and is readily removed by sweat. To secure water repellency various greases, fats, and oils were incorporated with the bentonite gel. It was necessary to use sodium lauryl sulfate in order to secure a smooth, uniform mixture when the fats were added to the gel. The materials added produced the desired water resistance but left a sticky film on the skin. Next, various pigments such as zinc oxide, magnesium oxide, magnesium carbonate, titanium dioxide, magnesium stearate, etc., were tried as fillers. These agents serve to overcome, to a certain extent, the after sticky or greasy feel. They also improve the water resistance, covering power, and screening power in a mechanical way. Titanium dioxide proved to be the best. Magnesium stearate improved water resistance and adherence to the skin. Mineral oil was used for water resistance, and, with diethylphthalate, gave a smoother texture to the ointment. The finished formulas Nos. 17D and 41A (Table I) have many of the desirable properties of the ideal preparation.

Several formulas were developed using methyl cellulose as the film-forming agent. Many of the desired properties of the ideal ointment were achieved except that of water resistance. It was found impossible, with the modifications tried, to waterproof the film left on the skin without having an excessively greasy feel and a tendency to roll when rubbed. The best formula is listed as No. 52 in Table I.

Sodium alginate (Kelgin) and pectin, both of which are film-forming agents, were next tried but were found unsuitable as bases. They were incompatible with the sunscreens and it was not possible to formulate a combination which would leave a water-resistant film. A keratin, prepared from duck feathers, was found to produce a good flexible film from a water solution (gel), but it could not be rendered resistant to sweat. Various resins, gum resins, and balsams were investigated but in no case did they materially improve the desired properties of any combination in which they were used.

Polyvinyl alcohol forms gels of varying consistency when mixed with water. The film left by PVA mixtures was rapid drying and flexible, but could not be waterproofed by oil or grease without rolling when applied to the skin. A representative formula, No. 43, given in Table I, does not meet the requirements of a satisfactory base. It is possible that by treatment of the PVA with a dibasic organic acid an ester with the desirable properties might be obtained, but the experiment was not tried. Vinyl resin (Vinylite), another plastic, is soluble in acetone and the film left from a solution is very tough and insoluble in water, but it is incompatible with the homomenthyl salicylate and with other ingredients required to compound a suitable ointment.

Ethyl cellulose (Ethocel) is soluble in alcohol and the resulting film is tough and insoluble. It could not be formulated as an ointment because of the rapid evaporation of the alcohol, and, when oily materials were added, the film remained tacky. A good lotion was prepared which has all the desired requirements, except that it is very hard to remove. The film left on the skin is quite permanent, resisting washing with soap and water but coming off readily with alcohol. The formula is given under No. 62A in Table I. A lotion using tincture of benzoin as the film-forming material was also tried and is listed as No. 101 in Table I. This lotion has many good properties but subjects object to the odor so much that it can hardly be considered useful.

TABLE I.—COMPOSITION OF THE OINTMENTS AND LOTIONS

| Compounds | Ointment Number | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 17D | 41A | 43 | 50 | 50A | 50B | 50C | 52 | 58 | 58D | 58E | 62A | 101 |
| Homomenthyl salicylate | 10 | 20 | 20 | 20 | 20 | .. | 20 | 20 | 20 | .. | 20 | 20 | 20 |
| Ethyl *p*-aminobenzoate | .. | .. | .. | .. | .. | 2 | 2 | .. | .. | 2 | 2 | .. | 2 |
| Titanium dioxide | 10 | 10 | 10 | 10 | 25 | 15 | 21 | 10 | 38 | 40 | 36 | 10 | 10 |
| Magnesium stearate | 10 | 10 | 10 | 5 | 12.5 | 20 | 12.5 | 5 | 20 | 20 | 20 | .. | .. |
| Butyl stearate | .. | .. | .. | 5 | 12.5 | 20 | 12.5 | 5 | 20 | 20 | 20 | .. | .. |
| Mineral oil | 10 | 5 | 5 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Diethylphthalate | 10 | 5 | .. | .. | .. | .. | .. | .. | .. | 16 | .. | .. | .. |
| Sodium lauryl sulfate | 0.25 | 0.3 | 1.0 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Bentonite ointment[a] | 47.75 | 47.7 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Pigment base[b] | 2.0 | 2.0 | .. | 2.0 | 2.0 | 1.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Vanishing cream[c] | .. | .. | .. | 58 | 28 | 37 | 30 | .. | .. | .. | .. | .. | .. |
| Other constituents | .. | .. | Kaolin 20, PVA[d] 20, water 14 | .. | .. | Carbitol 5 | .. | Methyl[e] cellulose 58 | .. | .. | .. | Talcum 20, Ethyl[f] cellulose 48 | Glycerin 8, tincture of benzein 58 |

[a] Bentonite 15%, distilled water 85%.
[b] Made up of burnt umber 50%, red ferric oxide 25%, yellow ferric oxide 25%.
[c] Made up of stearic acid 15%, cetyl alcohol 1%, triethanolamine, 1.5%, carbitol 10%, distilled water 72.5%.
[d] 5% polyvinyl alcohol in water.
[e] 5% methyl cellulose in water.
[f] 4% ethyl cellulose in alcohol.

In subsequent studies attempts were made to modify some of the bases already in use. Greasy or oily ointments are quite resistant to water and sweat but the film on the skin remains more or less sticky. Aquaphor, petrolatum, wool fat, mineral oil, and a number of vegetable oils in various combination with pigments to overcome the excessive greasiness yet remain water repellent proved unsatisfactory. In the combinations tried it was not possible even with low percentage of base content to retain water resistance yet eliminate the after-sticky feel. There was little difference between anhydrous and W/O emulsion types. One fatty material—stearate mixture—proved highly satisfactory as an ointment base and sunscreen vehicle and the formulas of the 58 series in Table I are of this type. The preparation was made of equal parts of magnesium stearate and butyl stearate, and had added to it the sunscreens, some titanium dioxide, and the pigments for matching skin color. In modification 58D diethylphthalate was added. This preparation resists water and sweat, gives a uniform film which is practically nonsticky and is quite resistant to rubbing with light clothing, and leaves the least after-sticky feel of all the combinations tried.

Another group of relatively successful preparations was made by using vanishing cream as a vehicle for the sunscreen. These are the No. 50 series in Table I. Since these also contain magnesium and butyl stearate as well as titanium dioxide, they are modifications of formula No. 58.

**Preparation of Ointments.**—All ointments can be prepared by simple admixture and no special sequence of procedures is necessary. For small quantities it is most convenient to use a spatula and glass slab.

The bentonite ointment base (17D and 41A) is prepared by mixing the bentonite with the water in a mortar. When all the water has been taken up the lumpy mass is transferred to a glass slab and rubbed smooth with a spatula. It is almost impossible to obtain a smooth mixture in the mortar alone.

The magnesium stearate–butyl stearate mixture (in the 50, 52, and 58 series of ointments) is prepared on a slab. Any amount of this mixture can be prepared at one time and used as a stock mixture. It saves considerable time in compounding.

The vanishing cream base (in the 50 series of ointments) is prepared in the usual manner. The stearic acid and cetyl alcohol are mixed in one dish and melted on a water bath; to this is added the triethanolamine, carbitol, and water mixture heated to the same temperature and the mixture is stirred until emulsified.

In each and all of the cases the sunscreens can be added by simple admixture at any time. Homomenthyl salicylate is a viscous fluid and can be added to base or pigment. Ethyl *p*-amino benzoate may be dissolved in alcohol or glycerin, if these are used in the preparations, or powdered more finely before incorporation with pigments or base.

**Tests of the Preparations.**—In order to test the sweat, water, and abrasion resistance of ointments it is necessary to compare the protective value of an ointment in some objective way immediately upon application and after subjecting it to sweating and abrasion. The basic unit accepted for this purpose and used in the table of data is the ratio of the time required to produce a minimal erythema through the ointment with the time required on control patches of skin. The minimal erythema dosage (at a fixed intensity used in all the experiments) is the light exposure which causes a just perceptible reddening of the skin in from four to twenty hours after irradiation. While the minimal erythema dosage varies from individual to individual depending on previous exposure, type of skin, etc., it is rather constant for a given area of skin of an individual.

For these determinations the skin of the arm or the abdomen was exposed through apertures cut in thin lead or adhesive tape. Flexible lead plates in various sizes from 2 in. by 4 in. to 4 in. by 10 in. with circular holes about $^{7}/_{16}$ in. in diameter punched through them were found the most satisfactory devices after trials with various types of adhesive, paper, and leather. The lead plates can be washed easily and sterilized between use by different subjects; they can be easily taped in place and molded to fit the arm or abdomen. A paper shield is taped to the lead plate to protect the surrounding portion of the body.

For irradiation a quartz mercury arc was used. The light was first filtered through 3 inches of water in a quartz flask then through a 2-mm. Corning Pyrex 9700 filter. This filter removes the short ultraviolet although it transmits some of the light at 2800 Å. which is not present in sunlight. Since this wave length lies near a region of minimal erythemal efficiency, the transmission does not seriously affect the results (2). The carbon arc gives a wavelength distribution in the ultraviolet much more like sunlight than the quartz mercury arc. However, not only was it difficult to maintain constancy of operation but the intensity was so much smaller that it tried the patience of the subjects. With the mercury arc an erythema could be produced in from one-half to two minutes on the unprotected skin of the various subjects tried.

The most efficient sunburn ointment or lotion is one which in thinnest layers protects the skin most effectively. Since the thickness of the layer cannot be measured it is necessary to spread a given weight of the preparation over a given area of skin. In preliminary trials it was found that 200 mg. of ointment per 52 cm.² of skin was too thick and some ointments began to roll off on rubbing. Therefore, tests were carried out with 150, 100, and 50 mg. per 52 cm.² To facilitate even application over the area the ointment was first daubed over the skin, then rubbed in to what appeared an even layer. There is an obvious source of error in this procedure but no better one was found. The results are given in Table II. Ointments 50 and 58 take a high place in this test. The two lotions, 62A and 101, were also tried but in this case the amount applied to the skin cannot be estimated. The protection of a layer as applied lightly with a swab compares favorably with the ointments, being 29 minimal erythemal units for No. 62A and 28 minimal erythemal units for No. 101.

TABLE II.—COMPARISON OF THE EFFICIENCY OF OINTMENTS OF DIFFERENT THICKNESS. EFFICIENCY MEASURED BY THE RATIO OF TIME TO PRODUCE A MINIMAL ERYTHEMA WITH OINTMENT TO TIME WITHOUT OINTMENT

| Ointment | Amount in Mg. per 52 Cm.² 50 | 100 | 150 |
|---|---|---|---|
| 41A bentonite | 4 | 4 | 16 |
| 50A vanishing cream stearate mixture | 7 | 9 | 16 |
| 50C vanishing cream | 7 | 15 | 25 |
| 58 stearate mixture | 5 | 11 | 20 |
| 58E stearate mixture | 9 | 18 | 23 |
| Commercial No. 1 | 2 | 3 | 19 |
| Commercial No. 3 | 3 | 9 | 24 |

The sweat resistance of the preparations is tested in the following manner: The subject is encouraged to exercise with weights for a few minutes before he is admitted to an insulated chamber kept at approximately 112° F. and at about 85% relative humidity. He is encouraged to drink hot tea with sugar and is given a salt tablet at the beginning of the test. Usually a drenching sweat is induced within ten to twenty minutes and this generally continues until the hour is up. As a measure of the relative sweating of different individuals or of the same individual at different times the forearm is wrapped in gauze and the wet weight compared with the dry weight. While crude, this method at least serves to indicate whether the individual test is satisfactory. The pulse rises from 10% to 50% and the temperature often rises a degree during the test.

The sweat was found to pass quite freely through the ointments and lotions tried, the sweating skin having much the same appearance in both coated and control areas. It is possible that sweating is somewhat reduced by the preparations but the methods did not allow of sufficiently accurate measurement. The results are given in Table III. It is apparent that in some cases the loss in protection is very great. Lanolin and aquaphor ointments are relatively useless after a sweat, as are also the commercial ointments tried. The bentonite, vanishing cream, and stearate ointments and the Ethocel and benzoin lotions remain effective even after the sweat. The values given in the table are the averages of 3 to 11 satisfactory trials. More experiments were performed but for various reasons the data could not be used. The degree of sweating even under what appeared to be the same conditions varied and with this the degree of protection varied as well. Thus subject "D" after the most profuse sweat recorded showed a loss in value of 21 units for both ointment 58E and lotion 62A (29 to 8 minimal

erythemal units). The experiments are inadequate to do more than indicate the order of change.

TABLE III.—SWEAT AND WATER RESISTANCE OF VARIOUS OINTMENTS (150 MG. PER 52 CM.²). THE VALUES ARE THE RATIOS OF THE TIME FOR MINIMAL ERYTHEMA WITH THE OINTMENT TO THE TIME WITHOUT THE OINTMENT

| Ointment | Before Treatment | After Sweating | After Washing |
|---|---|---|---|
| 41A bentonite | 18 | 12 | 4 |
| 50A vanishing cream and stearate mixture | 22 | 13 | 9 |
| 50C vanishing cream and stearate mixture | 29 | 19 | 10 |
| 58 stearate mixture | 23 | 15 | 13 |
| 58E stearate mixture | 29 | 19 | 11 |
| 38C lanolin-petrolatum | 15 | 3 | .. |
| 39 aquaphor mineral oil | 16 | 3 | .. |
| 62A Ethocel lotion | 29 | 25 | 11 |
| 101 tincture of benzoin | 28 | 16 | .. |
| Commercial No. 1 | 24 | 4 | 1 |
| Commercial No. 2 | 19 | 6 | 8 |

The manner in which sweating decreases the protective value of the ointments is unknown. The sweat coming through the ointment when caught on gauze is not colored; therefore presumably the ointment is not being carried off physically in large enough quantities to detect in this manner. It is possible that the sunscreen is selectively removed.

In Table III are given also the data for water resistance of the various preparations. In these experiments a stream of warm water was allowed to play for fifteen minutes over the covered area with the force of an ordinary shower. The results indicate that the bentonite ointment (41A) is less effective than the vanishing cream or stearate mixture type. In all cases, however, the water resistance is less than the sweat resistance. Attempts to compare the amount of material remaining in equal areas before washing and after were not conclusive, owing to the difficulty in scraping off the material.

The abrasion resistance of three of the preparations most effective in the preceding tests was next compared; namely, the vanishing cream preparation, 50C, the stearate preparation, 58E, and the Ethocel lotion, 62A. In these experiments the usual amount of the ointment 150 mg./52 cm.² or lotion was applied to the abdomen and the area was covered with a gauze bandage firmly taped in position. The subject carried on his usual activities and slept with the bandage. On the following day the area was irradiated in the usual way. It was observed that even after so long a time a fair degree of protection was still retained. Thus for 50C and 62A it was reduced from 29 to 10 minimal erythemal units, for 58C from 29 to 14. Again the number of cases is small so that only the order of the effect is indicated rather than absolute values. The loss of protection in the case of the ethocel lotion is surprising since this lotion forms a firm film which resists rubbing. No visible scaling resulting in a thinner film was noted.

## SUMMARY

1. An ideal sunburn preventive preparation should protect the skin in thin films, be easy to apply, dry readily, and leave no after-sticky or greasy feel, resist moisture, remain stable, be nonirritating and nonsensitizing, and wash off with soap and water.

2. New vehicles were studied. Of some 100 formulations tried, bentonite clay ointment, a stearate mixture base ointment, a vanishing cream, and an ethocel lotion, nearly all containing homomenthyl salicylate as a sunscreen proved most satisfactory. None is an ideal sunburn preventive.

3. As a pigment, titanium dioxide was found more satisfactory than magnesium oxide. The pigment gives covering power and mechanical screening.

4. While it is unlikely that any of the ingredients of these formulas would produce irritation upon continued and prolonged use, this possibility was not investigated.

5. Any of the formulas can be prepared with ordinary laboratory equipment on a small scale and, with slight modifications, in some cases they are suitable for manufacture on a large scale.

6. The value of an ointment as a sunburn protection is determined by comparing the ratio of the dosage required to produce a minimal erythema through the ointment by the dosage required in the control patch of skin (3).

7. A number of new preparations so evaluated show a high degree of sunburn protection.

8. Sweating decreases the protective value of these preparations but not nearly so much as it does some of the commercial preparations tried.

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# Studies on the Pharmacology of Aluminum Cholate*

By LLOYD W. HAZLETON and ANNA S. RILEY†

**The acute and chronic toxicities and cathartic activity of aluminum cholate was determined in mice. The $LD_{50}$ is about 100 mg. per mouse; the cathartic dose is about 30 mg. per mouse. After ninety days' feeding, no tolerance to the cathartic activity was noticed and no astringency from the aluminum was evidenced. The cathartic activity was the same order of magnitude as for sodium cholate indicating that the activity resides in the cholic acid portion and not in the aluminum cholate *per se*. Subacute feeding experiments show a definite toxicity at levels of 1.5 cathartic doses or higher daily.**

THE COMPOUND used in these studies was the aluminum salt of cholic acid prepared by the White Laboratories, Inc. Aluminum cholate is a white, water-insoluble compound which is soluble in dilute alkali. No previous investigations on this compound have been found in the literature. The following report is limited to studies on the toxicity and cathartic properties of the compound.

## EXPERIMENTAL PROCEDURES

**Acute Toxicity.**—The acute oral toxicity of aluminum cholate for mice is presented in Table I. A suspension of aluminum cholate in water was used in these studies and it was necessary to use 1-cc. volumes for administration of the larger doses. Although there is an occasional death at lower doses, the $LD_{50}$ for mice weighing 15 to 30 Gm. is approximately 100 mg. per mouse. In addition to the deaths tabulated there were a few latent deaths which, following autopsy, showed large amounts of aluminum cholate retained in the stomach of the mouse and it seems probable that such deaths are due to the physical characteristics of the compound rather than inherent toxicity. In view of the insolubility of the compound and the fact that solution in alkali results in the formation of an insoluble aluminum salt, toxicity following other routes of administration was not determined.

**Chronic Toxicity.**—To investigate the chronic toxicity of aluminum cholate a series of subacute feeding experiments was conducted. The series consisted of four groups of 20 male mice each, in cages with floors elevated above the droppings. They were allowed water and commerical powdered dog meal at all times. One group served as the control while aluminum cholate was added to the food of the other three groups in such quantity that the anticipated daily intake of food would contain 0.5, 1.0, and 2.0 cathartic doses (see below) of aluminum cholate daily. Experiments were continued for ninety days. Growth and mortality curves are presented in Fig. 1 while food and aluminum cholate intake are presented in Table II. It will be noted that in the 0.5 and 1.0 cathartic dose groups the actual intake exceeded the expectations, i.e., 23 vs. 15 and 44 vs. 30 mg. in these groups, respectively. The mice at the 2.0 cathartic dose level refused the aluminum cholate food and the actual intake of aluminum cholate was not as great as in the 1.0 dose level although the mortality rate was very high. It would appear that starvation played an important role in these deaths. Evaluation of the results indicates that the ingestion of 23 mg. of aluminum cholate per day, or about two-thirds the cathartic dose, has no deleterious effects whereas ingestion of 44 mg. or approximately 1.5 cathartic doses per day somewhat retards the growth and increases the mortality rate. The mechanism of this chronic toxicity has not as yet been established.

TABLE I.—TOXICITY AND CARTHARTIC RESPONSE FOLLOWING ORAL ADMINISTRATION OF ALUMINUM CHOLATE TO MICE OF WEIGHT 15–30 GM. (LATENT DEATHS ARE NOT INCLUDED)

| Dose | | No. of | Observed Data | | Integrated Data | | | No. of | Per Cent |
|---|---|---|---|---|---|---|---|---|---|
| Mg./Mouse | Cc. | Mice | No. Dead | % Dead | Dead | Living | % | Positive | Positive |
| 20 | 0.5 | 29 | 0 | 0 | .. | 306 | .. | 10 | 34 |
| 30 | 0.5 | 67 | 2 | 3 | 2 | 277 | .. | 30 | 46 |
| 40 | 0.5 | 109 | 11 | 10 | 13 | 232 | 5 | 56 | 57 |
| 45 | 0.5 | 28 | 1 | 4 | 14 | 134 | 10 | 17 | 63 |
| 50 | 0.5 | 51 | 9 | 18 | 23 | 107 | 18 | 38 | 90 |
| 75 | 0.5 or 1.0 | 35 | 5 | 14 | 28 | 65 | 30 | 26 | 87 |
| 100 | 0.5 or 1.0 | 38 | 14 | 37 | 42 | 35 | 54 | 22 | 92 |
| 125 | 1.0 | 25 | 18 | 72 | 60 | 11 | 84 | 6 | 86 |
| 150 | 1.0 | 25 | 21 | 84 | 81 | 4 | 95 | 4 | 100 |

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During the experiments the mice of all groups including controls showed a slight degree of catharsis and at no time was there evidence of tolerance to the cathartic action of aluminum cholate.

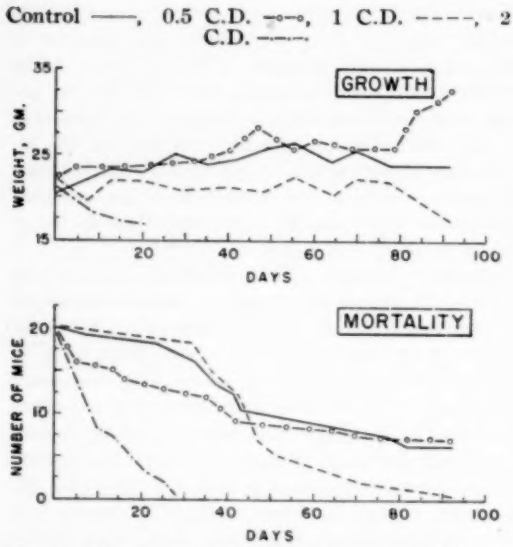


Fig. 1.—Growth and Mortality Curves of Mice on Diets Containing Aluminum Cholate.

based on surviving mice only, are summarized in Table I. The cathartic dose (for 50% of the animals) is approximately 30 mg. per mouse. This value is equivalent to 150% of the cathartic dose for cholic acid and to 120% of the calculated cathartic dose for aluminum cholate. At no time was there any evidence of an astringent action as might be anticipated in the presence of an aluminum salt. Following the large doses catharsis generally preceded death.

## DISCUSSION

It has been previously shown (2) that sodium taurocholate and sodium glycocholate do not produce catharsis in nontoxic doses and that cholic acid in the form of its sodium salt does produce catharsis without appreciable toxicity. Although the aluminum salt differs from the sodium salt in that it is insoluble and contains the astringent aluminum ion, its cathartic activity approximates that of sodium cholate and its toxicity is of the same order of magnitude.

It has been reported (3) that mice receiving 20 mg. of bile salts compound daily, approximating one-half the cathartic dose,

TABLE II.—FOOD AND ALUMINUM CHOLATE CONSUMPTION OF MICE

| Group | Duration | | Food Consumption | | Aluminum Cholate | | Number of Mice | |
|---|---|---|---|---|---|---|---|---|
| | Days | Mouse Days,[a] | Total, Gm. | Daily Av. per Mouse, Gm. | Total, Mg. | Daily Av. per Mouse, Mg. | Start | Finish |
| Control | 92 | 1184 | 5251 | 4.4 | .... | .. | 20 | 6 |
| 0.5 C. D.[b] | 92 | 1020 | 4778 | 4.6 | 23,890 | 23 | 20 | 7 |
| 1 C. D. | 92 | 979 | 4347 | 4.4 | 43,470 | 44 | 20 | 0 |
| 2 C. D. | 28 | 248 | 403 | 1.6 | 8,060 | 32 | 20 | 0 |

[a] Mouse days—number of mice, adjusted with each mortality, times the number of days.
[b] C. D.—cathartic dose, which is 30 mg. for aluminum cholate.

**Cathartic Acitivity.**— *In vitro* studies indicate that aluminum cholate should pass through the stomach unchanged but form an alkaline cholate and an aluminum salt in the intestine. *In vivo*, therefore, it might be expected that aluminum cholate would possess cathartic activity similar to sodium cholate.

The assay method of the U. S. P. XII (1) for the cholic acid equivalent of extract of bile was used to determine the equivalent for aluminum cholate. The results indicate that each milligram of aluminum cholate is equivalent to 0.8 mg. of cholic acid. Since the cathartic dose for cholic acid as the sodium salt is 20 mg. per mouse, the theoretical cathartic dose for aluminum cholate would be 25 mg. per mouse.

The cathartic activity of aluminum cholate in mice was determined by methods previously used in this laboratory (2). The results of these tests, weighed slightly less and had a higher mortality rate than the control animals. Figures are not available for subacute feeding of cholic acid itself. It is difficult to evaluate the significance of the chronic toxicity following aluminum cholate as described above but such evidence as is available indicates that the phenomenon is characteristic of bile salts rather than of aluminum cholate *per se*.

The cathartic activity substantiates the *in vitro* observations on the breakdown of aluminum cholate. Comparisons of the choleretic activity of sodium cholate and aluminum cholate are in progress and will be reported later. Studies directed toward

further investigation of the enteric-like properties of aluminum cholate appear to be desirable.

## CONCLUSIONS

1. The cathartic dose of aluminum cholate in mice is approximately 30 mg. per mouse.
2. The oral $LD_{50}$ is approximately 100 mg. per mouse.
3. No evidence of tolerance to cathartic action of aluminum cholate was apparent after ninety days' feeding of the compound.
4. Subacute feeding experiments indicate that a definite toxicity accompanies an intake of approximately 1.5 cathartic doses daily.

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# Determination and Stability of Menadione*

By WILLIAM R. COLLINS and ERNST R. KIRCH

**The reaction for the determination of 2-methyl-1,4-naphthaquinone using 2,4-dinitrophenylhydrazine has been modified to improve its sensitivity without loss of accuracy. The stability of menadione toward diffuse daylight has been investigated.**

MENADIONE and its water-soluble derivatives have been introduced to the medical profession as therapeutic agents which possess vitamin K activity. Increased interest in these substances has necessitated the development of a convenient and accurate method for their determination and the investigation of the stability of the compounds to light.

It is known that vitamin $K_1$ is affected by light with loss of physiological activity and modification of the absorption spectrum (1). It has also been shown that vitamin $K_1$ in purified hexane is stable for periods up to five months when stored in the dark at room temperature (2). MacCorquodale, *et al.* (3), reported that vitamin $K_2$ is unstable when exposed to light. Ewing, Vandenbelt, and Kamm (4) have shown a deterioration of the absorption curves when samples of vitamin $K_1$ and $K_2$ were exposed to diffuse light. 2-Methyl-1,4-naphthaquinone upon exposure to light for long periods has been shown to decolorize with the formation of a polymer of unknown structure (5).

Of the several procedures (6–14) for the estimation of menadione the method of Menotti (10) seems to be the most promising one. The determination is based on the sensitive color test for 2-methyl-1,4-naphthaquinone and related substances as reported by Novelli (9). It depends on the formation of the 2,4-dinitrophenylhydrazone of the naphthaquinone and the interaction of the latter compound with ethanolic ammonia to yield a green-blue colored solution. The green color is said to be due to a combination of a blue color exhibited by the 2,4-dinitrophenylhydrazone in the alcoholic ammonia with the yellow color of the 2,4-dinitrophenylhydrazine, which is in excess.

The method as developed by Menotti was limited to quantities of not less than 50 γ. We have been able to increase the sensitivity tenfold.

Samples of 2-methyl-1,4-naphthaquinone and its bisulfite derivative were exposed to diffuse light for various periods of time and in various concentrations in order to determine the destruction by light.

* Received Jan. 25, 1946, from the Dept. of Chemistry, College of Pharmacy, University of Illinois, Chicago.

## EXPERIMENTAL

**Reagents.**—Stock solution: Dissolve 100 mg. of 2-methyl-1,4-naphthaquinone (m. p. 105–107°) in 200 ml. of 95% alcohol. This solution is stable if protected from light.

When using menadione sodium bisulfite[1] an equivalent amount is dissolved in about 50 ml. of distilled water and then brought to volume (200 ml.) with alcohol.

Aliquots of these solutions are diluted with alcohol to the desired concentration.

Dinitrophenylhydrazine Reagent: 125 mg. of 2,4 dinitrophenylhydrazine (E. K. Co. #1866) is dissolved in 50 ml. of 2 *N* hydrochloric acid.

Alcoholic Ammonia: Ammonium Hydroxide (sp. gr. 0.90) is mixed with an equal volume of 95% alcohol.

**Method.**—Aliquots of alcoholic solutions representing from 5 to 40 γ of 2-methyl-1,4-naphthaquinone are transferred to 50-ml. volumetric flasks. One milliliter of the 2,4 dinitrophenylhydrazine reagent is added and the flasks are heated for fifteen minutes in a water bath of boiling water. After heating, cool the reaction mixture to room temperature, add about 15 ml. of 95% alcohol, follow 5 ml. of the alcoholic ammonia and enough alcohol to volume. After twenty minutes the maximum color develops and is stable for about five hours. The color is read in a spectrophotometer at 635 mμ and follows the Beer–Lambert law. For the blank the menadione is omitted. Figure 1 shows a typical absorption curve.

Menadione sodium bisulfite gives the same color reaction and can be determined by this procedure calculating the results from a curve developed for the menadione.

**Stability of Menadione.**—Alcoholic solutions containing various concentrations of 2-methyl-1,4-naphthaquinone were exposed to daylight using containers consisting of different types of glass. Ordinary pyrex flasks were used as containers for group one, pyrex flasks "low actinic" for the second group and brown bottles for group three. Controls for each group were kept in the dark during the time of investigation. While no record was made of the intensity of the light on various days, it was noted that, so far as the samples of group one are concerned, there was an 80% destruction of the menadione within five hours (Table I). Solutions kept in "low actinic" pyrex flasks or in brown bottles showed no destruction. Controls kept in the dark assayed the same concentration of menadione as that of the solutions prior to standing in the dark.

Menadione sodium bisulfite remained stable under comparable conditions.

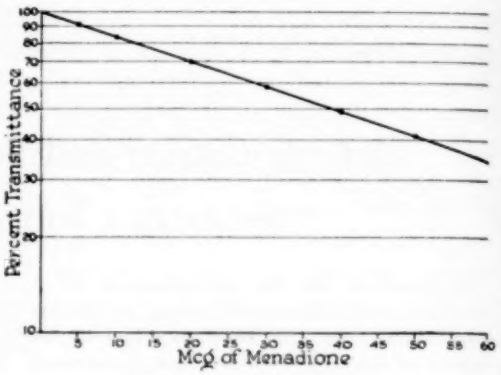


Fig. 1.—Standard Curve.

## DISCUSSION

Strong alkalies such as potassium or sodium hydroxide in place of the ammonium hydroxide have been found to lead to an extensive decomposition of the 2,4-dinitrophenylhydrazine and to the formation of colored substances which interfere with the color comparison. Ammonium hydroxide in a final concentration of 0.5 *N* was found to be necessary to bring about conversion of

TABLE I.—DESTRUCTION OF MENADIONE BY LIGHT

| Concentration of Menadione, Mg./Ml. | 0 Hr. | | 1 Hr. | | 2 Hr. | | 3 Hr. | | 3.5 Hr. | | 5 Hr. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mg. Found | % Destroyed | Mg. Found | % Destroyed | Mg. Found | % Destroyed | Mg. Found | % Destroyed | Mg. Found | % Destroyed | Mg. Found | % Destroyed |
| 500 | 505 | 0 | 375 | 25 | .. | .. | 275 | 45 | .. | .. | .. | .. |
| 250 | 249 | 0 | .. | .. | .. | .. | .. | .. | 87.5 | 65 | .. | .. |
| 100 | 99 | 0 | .. | .. | .. | .. | 43 | 57 | .. | .. | .. | .. |
| 100 | 102 | 0 | 74 | 26 | 62 | 38 | 52 | 48 | 36 | 64 | 19 | 81 |
| 100 | 99 | 0 | 78 | 22 | 64 | 36 | 54 | 46 | 38 | 62 | 21 | 79 |
| 50 | 50 | 0 | .. | .. | 30 | 40 | .. | .. | .. | .. | 10 | 80 |
| 35 | 35 | 0 | .. | .. | .. | .. | 19 | 44.5 | .. | .. | .. | .. |
| 30 | 29 | 0 | .. | .. | 17 | 42 | .. | .. | .. | .. | 6.5 | 87 |
| 20 | 20 | 0 | .. | .. | 12 | 40 | .. | .. | .. | .. | 3.0 | 85 |
| Average | .. | .. | .. | 24.3 | .. | 39.2 | .. | 48 | .. | 63.6 | .. | 82.4 |

[1] Hykinone was kindly furnished by the Abbott Laboratories, North Chicago, Ill.

the alcohol-insoluble hydrazone to the blue compound which is soluble in alcohol (10).

Heating the reaction mixture to a higher temperature resulted in an increase in sensitivity without loss of accuracy and reproducibility.

Diffuse daylight will destroy the vitamer rather rapidly. Although we did not measure the variation in intensity of the diffuse daylight on various days the experiment was conducted, it was found that the destruction due to light would increase extension of exposure time. Exposure of one hour resulted in a deterioration of 22 to 26 per cent, while at the end of the second hour about 40 per cent of menadione was destroyed. At the end of five hours about 80 per cent was bound to be destroyed. After five hours of exposure, the rate of deterioration seems to be less. In three experiments the solutions were allowed to remain exposed to daylight (summer) for one week. The maximum destruction at the end of that period was found to be about 90 per cent. The controls as well as the solutions in "light protecting" containers showed 100 per cent stability.

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# Studies on the Cutaneous Absorption of Insulin*

By JAMES C. H. RUSSELL,† L. W. BUSSE,‡ and ARTHUR H. UHL§

**The cutaneous absorption of insulin, using rabbits as test animals, has been studied. The results reported indicate that the application of standard insulin solutions and ointments containing insulin do not produce a lowering of blood sugar levels. Treatment of the skin with petroleum ether or chloroform prior to applying as solutions of insulin, however, is shown to result in a lowering of blood sugar.**

FOLLOWING the discovery of insulin, several investigators attempted to introduce it into the body by inunction. The reports of the possibility of insulin absorption through the skin are conflicting.

Woodyatt (1) in 1922 conducted experiments with oral, rectal, vaginal, and intranasal administration. He obtained "very weak, doubtful, or frankly negative results." In 1923, Rennie (2) found that the inunction of 100 units of insulin on a human diabetic had no effect. Telfer (3) reported that insulin could be introduced into the blood stream of rabbits by the inunction of insulin ointments, of which lard and lanolin were the bases, to the shaved skin of the abdomen. Because he used an unstandardized insulin, it was impossible to calculate the number of units employed. Harrison (4) in 1926 was unable to confirm Telfer's results. In 1935 Hermann (5) and Hermann and Kassowitz (6) reported a marked fall of blood sugar in animals and patients following the application of an insulin salve to the skin. Major (7) in 1935 and 1936 (8, 9) concluded that under proper conditions insulin could be

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Abstract from a part of the thesis submitted by J. C. H. Russell to the Graduate Faculty of the University of Wisconsin, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
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absorbed through the skin of rabbits. His procedure was to rub the shaved abdomen of the rabbits with 3 to 4 drops of glycerin and in ten to fifteen minutes the insulin preparation was applied. After application the solution was gently rubbed with a glass rod and care was taken to avoid injury to the skin. In 1936 Bruger and Flexner (10) came to the conclusion that: "The absorption of insulin by the skin of rabbits is dependent upon the integrity of the integument. The intact skin shows little or no absorption, whereas a recently abraded skin, such as produced by shaving, permits the absorption of an appreciable amount of insulin." Starkenstein and Hendrych (11) in the same year reported that insulin could be absorbed by the skin from an undisclosed ointment providing the cholesterol was first removed from the skin by some organic solvent such as petroleum ether. They also stated that saponin added to the preparation increased the absorption of the insulin. In 1937 Major and Delp (12) stated that their experiments showed that cutaneous absorption of insulin occurred in rabbits and in patients, independent of abrasions produced on the skin. They concluded, however, that the absorption was very inconstant.

## EXPERIMENTAL

Attempts were made to study the cutaneous absorption of insulin. Rabbits, weighing between 1.5 and 2.0 Kg., were used as test animals. Between experiments they were kept on a diet of hay and oats, but were not fed twenty-four hours previous to the experiments and received no food during the experiments. The hair of the abdomen was removed with an electric clipper. No vehicle was used for the standard solutions of insulin which were applied by inunction. Experiments were carried out using 20, 40, and 160 units of insulin. Three to five rabbits were used with each series. No marked lowering of the blood sugar was observed when determined by Folin's Micro-Method. Table I shows typically negative results obtained in this series.

The data from this series indicated that insulin was not absorbed through the skin in doses as high as 160 units.

A series of experiments was carried out using varying units of standard insulin solutions applied to the skin of rabbits after pretreating the skin with chloroform, petroleum ether, and ethyl alcohol. Starkenstein and Hendrych (11) postulated that insulin applied to the skin produced a marked lowering of the blood sugar, if applied after rubbing the skin with $CHCl_3$ and P. E., both solvents for cholesterol. Since no reports were observed in the literature showing the quantitative solubility of cholesterol in those solvents, it was thought to the point to carry out such an experiment. The results are shown in Table II. Typical results of the absorption of 80 and 160 units of insulin, when applied to the skin after pretreatment with chloroform, are shown in Table III.

TABLE I.—APPLICATION OF STANDARD INSULIN SOLUTION: NO PRETREATMENT

| Hours | Time | | Mg. % Glucose |
|---|---|---|---|
| | 8:50 a. m. | Normal..... | 93.02 |
| | 9:30 a. m. | Applied 160-U | |
| 1 | 10:30 a. m. | ..... | 97.56 |
| 2 | 11:30 a. m. | ..... | 95.24 |
| 3 | 12:30 p. m. | ..... | 95.71 |
| 4 | 1:30 p. m. | ..... | 96.50 |
| 5 | 2:30 p. m. | ..... | 93.89 |

TABLE II.—SOLUBILITY OF CHOLESTEROL (22°)

| | Gm./Cc. |
|---|---|
| In chloroform | 0.4819 |
| In petroleum ether | 0.0133 |
| In ethyl alcohol (95%) | 0.0084 |

These data indicated that it was possible to lower the blood sugar level by applying standard insulin solution to the abdomen of rabbits which was pretreated with chloroform. Two of the 11 rabbits in this series went into convulsions. There was a marked lowering of the blood sugar with the application of 80 and 160 units. Little or no lowering was observed with 20 units and 2 experiments with 40 units failed to show a marked drop in the blood sugar level.

Petroleum ether was then employed to pretreat the skin. Table IV shows the typical results from this series.

The results show that 20 units produced a pronounced drop in the blood sugar after the skin was pretreated with petroleum ether. It was then assumed that all units above 20 would go through the skin. One experiment was carried out using 80 units and one using 160 units. Both rabbits went into convulsions within two hours. These results indicated that petroleum ether was a more efficient agent in preparing the skin than was chloroform. These results did not seem to substantiate the theory proposed by Starkenstein and Hendrych (11) that cholesterol was the substance present in the skin which prevented absorption of insulin through it. If cholesterol were responsible for hindering absorption of substances through the skin, then chloroform should be a more efficient agent in preparing the

skin for the absorption of insulin than petroleum ether by virtue of its much greater solvent action on cholesterol than petroleum ether. (See Table II.)

TABLE III.—APPLICATION OF STANDARD INSULIN SOLUTION: CHLOROFORM PRETREATMENT

| Hours | Time | | Mg. % Glucose |
|---|---|---|---|
| | 1:00 p. m. | Normal | 100.00 |
| | 1:10–1:15 | Applied $CHCl_3$ | |
| | 1:15–1:20 | Applied 80-U | |
| 1 | 2:20 p. m. | | 89.65 |
| 2 | 3:20 p. m. | | 83.33 |
| 3 | 4:20 p. m. | | 70.43 |
| 4 | 5:20 p. m. | | 85.59 |
| 7 | 8:20 p. m. | | 101.09 |
| | 12:55 p. m. | Normal | 95.23 |
| | 1:00–1:05 | Applied $CHCl_3$ | |
| | 1:05–1:15 | Applied 160-U | |
| 1 | 2:15 p. m. | | 66.66 |
| 2 | 3:15 p. m. | | 74.07 |
| 3 | 4:15 p. m. | | 64.51 |
| 4 | 5:15 p. m. | | 45.45 |
| 6 | 7:15 p. m. | | 45.45 |
| | | Rabbit went into convulsions and was treated with glucose intravenously. | |
| | 12:45 p. m. | Normal | 108.11 |
| | 1:00–1:05 | Applied $CHCl_3$ | |
| | 1:05–1:15 | Applied 160-U | |
| 1 | 2:15 p. m. | | 69.68 |
| 2 | 3:15 p.m . | | 55.55 |
| 3 | 4:15 p. m. | | 54.94 |
| 4 | 5:15 p. m. | | 72.20 |
| 6 | 7:15 p. m. | | 95.79 |

TABLE IV.—APPLICATION OF STANDARD INSULIN SOLUTION: PETROLEUM ETHER PRETREATMENT

| Hours | Time | | Mg. % Glucose |
|---|---|---|---|
| | 9:30 a. m. | Normal | 105.26 |
| | 9:35–9:40 | Applied P. E. | |
| | 9:40–9:45 | Applied 20-U | |
| 1 | 10:45 a. m. | | 65.73 |
| 2 | 11:45 a. m. | | 71.68 |
| 3 | 12:45 p. m. | | 76.33 |
| 4 | 1:45 p. m. | | 102.56 |
| 5 | 2:45 p. m. | | 104.61 |
| | 9:00 a. m. | Normal | 114.28 |
| | 9:15–9:20 | Applied P. E. | |
| | 9:20–9:30 | Applied 80-U | |
| 1 | 10:30 a. m. | | 58.11 |
| 2 | 11:30 a. m. | Rabbit went into convulsions | |
| | 1:30 p. m. | Normal | 102.56 |
| | 1:55–2:00 | Applied P. E. | |
| | 2:00–2:10 | Applied 160-U | |
| 1 | 3:00 p. m. | | 35.08 |
| 1¾ | 3:45 p. m. | Rabbit went into convulsions | |

In the next series of experiments, ethyl alcohol, 95%, was employed to pretreat the skin. The results showed that this agent failed to exert a beneficial effect and did not allow the penetration of the insulin solutions.

The skin area pretreated with petroleum ether and chloroform became scaled, crusted, and desquamated several days after the experiments. This condition did not improve during a period of two weeks. The skin area pretreated with alcohol was not changed to any great degree and after several days appeared normal.

Starkenstein and Hendrych reported that 20 units of insulin incorporated in a C-16 alcohol ointment produced a startling drop in a rabbit's blood sugar level if the skin was first treated with petroleum ether. They did not disclose the formula of their ointment or the type of the insulin—solution or solid. A base was then prepared consisting of cetyl alcohol 8 parts, aerosol 0.5 part, and petrolatum 26.5 parts. One cubic centimeter of a standard insulin solution containing 20 units per cc. was incorporated with 2 Gm. of this base and applied to the shaved abdomen of a rabbit. This produced no effect and similar negative results were obtained with 40 units.

Two cubic centimers of U-80 insulin solution, 160 units, was incorporated with 4 Gm. of aquaphor and this preparation applied to the clipped abdomen of a rabbit pretreated with chloroform.

Another preparation (160 units) was then rubbed on the abdomen of another rabbit pretreated with petroleum ether.

Neither experiment produced a lowering of the blood sugar. These results indicated that the ointments used above hindered absorption of the insulin.

One disadvantage in the cutaneous administration of insulin is that the duration of action is only from five to seven hours. Experiments are now being carried out in which insulin is incorporated in a base consisting essentially of Stibnite and Bloodroot. This base is employed by Dr. Frederic E. Mohs, Assistant Professor of Chemosurgery and Research Associate in Cancer Research at the University of Wisconsin, who suggested the use of this combination. Preliminary work indicates that the duration of action of the insulin from this preparation may be ten to twelve hours or more.

## SUMMARY

1. Standard insulin solutions, alone, in doses as high as 160 units failed to lower the blood sugar level after their application to the abdominal skin of rabbits.

2. It was found that if the skin was first treated with chloroform or petroleum ether, and standard solutions of insulin applied, a lowering of the blood sugar resulted.

3. This effect was not observed in experiments in which 95% ethyl alcohol was employed to treat the skin.

4. Standard insulin solutions incorporated in ointment bases failed to lower the blood sugar level after their application to the abdominal skin of rabbits either with pretreatment or without. This indicates

that ointments tend to hinder rather than promote the absorption of insulin.

5. The skin of the rabbits became scaled, crusted, and desquamated from the treatment with chloroform and petroleum ether, but not from the alcohol. This indicated that these solvents removed fatty material from the skin and allowed penetration to take place.

6. It was reported that cholesterol was the substance which prevented absorption. Since cholesterol was found to be more soluble in chloroform, the best absorption should be expected with the use of this substance. Such was not the case as the more pronounced effect resulted from the use of petroleum ether.

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# A Study of the Determination of Some Official Aldehydes*

By MARY ESTILL MARTIN, KENNETH L. KELLY, and MELVIN W. GREEN

**A study of volumetric oxime methods and gravimetric methods using 2,4-dinitrophenylhydrazine as a reagent was made on benzaldehyde, cinnamaldehyde, and vanillin. Vanillin, the purity of which was determined by a methoxy determination and melting point, was used as the reference compound. The method adopted for N. F. VIII gave the most satisfactory results.**

WHEN cinnamaldehyde was introduced into the Third Supplement of National Formulary VII, the official hydroxylamine method for the assay of benzaldehyde did not prove entirely satisfactory, and a modification was adopted (1). This method proved to be so satisfactory that its use was extended to the assay of benzaldehyde and bitter almond oil. In order to justify such a change a study of the determination of aldehydes was made in the laboratory.

Aldehydes and ketones and essential oils containing them have been determined by various methods. Although the procedure of forming an addition compound with sulfite or bisulfite and aldehydes or ketones in essential oils has been used, it has not proved to be completely satisfactory. Wherever possible it is advisable to check the results obtained by the bisulfite method with a cold hydroxylamine titration (2).

A gravimetric method, using phenylhydrazine as the precipitating agent, has been adopted as official for the assay of benzaldehyde in almond extract by the Association of Official Agricultural Chemists (3). However, 2,4-dinitrophenylhydrazine has been described as a more suitable precipitating agent because of the instability of phenylhydrazine, due to its tendency to oxidize (4). Since the gravimetric method using 2,4-dinitrophenylhydrazine has proved reasonably effective in the official method for the assay of Camphor Spirit in N. F. VIII, it was deemed advisable to include this method in our study of the determination of aldehydes.

From a survey of the literature available, it was fountl that a cold hydroxylamine titration for the determination of aldehydes was effective and popular. There are many adaptations and modifications in the use of hydroxylamine hydrochloride. It is applicable for the assay of aldehydes and ketones other than those in essential oils. "Further-

* Received April 24, 1946, from the A. PH. A. Laboratory, Washington, D. C.

more, it is susceptible to variations to meet individual requirements, e.g., as in the analysis of camphor" (5). Naves, in a study of sweet orange oil from French Guinea, concluded that the hydroxylamine hydrochloride method was the only one giving accurate results in the determination of aldehydes (6).

Since benzaldehyde and cinnamaldehyde are liquids and subject to volatilization and deterioration by oxidation, a solid with a sharp melting point and known purity was used as a standard in comparing methods studied in the laboratory. Vanillin, the purity of which was known from a previous methoxy determination made in the laboratory and which has a sharp melting point of 80–82°, was chosen.

## EXPERIMENTAL

### Hydroxylamine Methods

For convenience, the two hydroxylamine methods studied were designated as the N. F. VII method and the N. F. VIII method. The N. F. VII method is the one described for the assay of benzaldehyde in N. F. VII, and the N. F. VIII method is the procedure given for the assay of cinnamaldehyde in the Third Supplement to N. F. VII and more recently adopted as the official method of assay for benzaldehyde, cinnamaldehyde, and benzaldehyde in bitter almond oil in N. F. VIII.

Although the two methods appear to be similar, they differ basically in character of the reagent solution used. In the N. F. VII method the hydroxylamine hydrochloride in an alcoholic solution reacts with the aldehyde, liberating free hydrochloric acid. The liberated hydrochloric acid is titrated with sodium hydroxide. A blank containing the same quantities of reagents, but omitting the aldehyde, is run. To determine the number of cubic centimeters of the sodium hydroxide required to neutralize the liberated hydrochloric acid, the volume of base used in the blank is subtracted from the volume used in the sample. The reactions are as follows:

$$R{\cdot}CHO + H_2N{\cdot}OH{\cdot}HCl \rightarrow R{\cdot}CH{=}N{\cdot}OH + H_2O + HCl$$

$$HCl + NaOH \rightarrow NaCl + H_2O$$

In the N. F. VIII method the hydroxylamine reagent solution contains free hydroxylamine in an excess of base. The hydrochloric acid of the hydroxylamine hydrochloride has been neutralized with potassium hydroxide, and an excess of the potassium hydroxide is present. In the blank the free hydroxylamine acts as a base titratable with hydrochloric acid, as illustrated in the following equation:

$$H_2NOH + HCl \rightarrow H_2NOH{\cdot}HCl$$

The aldehyde in the sample reacts with the $H_2NOH$, reducing the amount of base, and the excess reagent is titrated with hydrochloric acid. To determine the volume of acid required for the aldehyde, the volume of acid used for the sample is subtracted from the volume used for the blank.

The same indicator, bromophenol blue, is used in both titrations, and the color of the end point is the same, although approached from different points on the *p*H scale. In the N. F. VII method, the greenish yellow color of the end point is approached from the yellow or acid side, while in the N. F. VIII method, the end point is approached from the blue or basic side. In the first method, the indicator is added to each titration separately; in the second method the indicator is incorporated in the hydroxylamine reagent solution.

**Vanillin.**—The sample of vanillin studied had been previously assayed by a methoxy determination.[1] The calculated value for methoxy group in vanillin is 20.41%; the determined per cent of methoxy group was 20.44%, which corresponds to 100.1% of vanillin. The melting point of the sample of vanillin studied was found to be 82.2°, corresponding closely to the official melting point of 80–82°.

TABLE I.—DETERMINATION OF VANILLIN BY HYDROXYLAMINE METHODS

| Weight of Sample, Gm. | Method | Weight of Vanillin Recovered, Gm. | Vanillin Recovered, % |
|---|---|---|---|
| 1.5010 | N. F. VII | 1.5250 | 101.60 |
| 1.5009 | N. F. VII | 1.5170 | 101.10 |
| 1.5004 | N. F. VII | 1.5184 | 101.20 |
| 1.5006 | N. F. VII | 1.5001 | 99.98 |
| 1.5002 | N. F. VII | 1.4930 | 99.55 |
| 1.5007 | N. F. VII | 1.4920 | 99.39 |
| 1.5010 | N. F. VII | 1.4880 | 99.18 |
| 1.5001 | N. F. VIII | 1.4995 | 99.98 |
| 1.5012 | N. F. VIII | 1.5061 | 100.30 |
| 1.5003 | N. F. VIII | 1.4980 | 99.90 |

The results of the assay of vanillin by the N. F. VII and the N. F. VIII methods are given in Table I.

In both methods there is some difficulty in determining the correct color of the end point and in matching the samples to the blank. There is also difficulty in titrating to different end points and keeping the ratio of the volume required for the blank to the volume required for the sample equal. However, with experience the difference in the ratio of the volume becomes negligible. Care and experience on the part of the worker will reduce the difficulty of matching the end-point color of the sample to that of the blank. That this is so can

[1] The authors are indebted to Miss Nancy Green of the A. PH. A. Laboratory for determining the purity of this sample of vanillin by a methoxy determination.

be seen by comparing the first three determinations with the latter ones (Table I).

In developing the N. F. VIII method, an electrometric titration, in which the various colors were compared to Munsell color charts, was made (see Fig. 1). From the electrometric titration curve, the end point was found to have a *p*H of 3.45 and to be a greenish yellow color. There may be some discrepancy in the results if the titration is carried to other colors in the yellow-blue range of the indicator because of the difference in the *p*H.

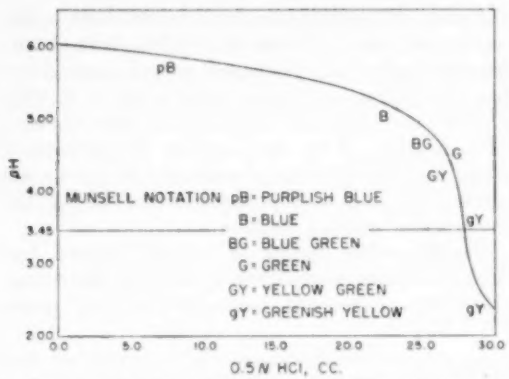


Fig. 1.—Electrometric titration curve of hydroxylamine reagent. Letters on curve refer to Munsell color names.

**Benzaldehyde.**—Benzaldehyde was assayed by the N. F. VII and the N. F. VIII methods. The results are given in Table II. It was soon noticed that samples titrated last gave higher results than those titrated first. Therefore, the standing period in both methods was increased to thirty minutes. The data in Table II show also the effect of time on the results.

**Cinnamaldehyde.**—When cinnamaldehyde was introduced into the Third Supplement to N. F. VII, a thorough study of the N. F. VIII method was made on this compound by one of us (K. L. K.). It was noticed, as in benzaldehyde, that too short a standing period gave low results (see Table III).

**Hydroxylamine Method Using Pyridine to Displace Oxime Equilibria.**—Bryant and Smith (7) modified the N. F. VII method, previously discussed, by using pyridine in the reagent for "the double purpose of forcing oxime formation to completion and of rendering the initial reaction mixture neutral to bromophenol blue, thus eliminating the need of a blank titration" (7). A laboratory study of this method was made using vanillin and benzaldehyde. The results are shown in Table IV.

From the data given in Table IV it is evident that the method suggested by Bryant and Smith gives a much lower per cent of recovery than either the N. F. VII or the N. F. VIII method. The lower results may be due in part to the fact that the color of the end point in this titration is greenish blue while the correct end-point color, with a *p*H of 3.45, is greenish yellow.

**2,4-Dinitrophenylhydrazine Method**

A study of a gravimetric method for the assay of these aldehydes, using 2,4-dinitrophenylhydrazine as the precipitating agent, was made also. Vanillin

TABLE II.—DETERMINATION OF BENZALDEHYDE BY HYDROXYLAMINE METHODS

| Weight of Sample, Gm. | Method | Period of Standing, Min. | Weight of Benzaldehyde Recovered, Gm. | Benzaldehyde Recovered, % |
|---|---|---|---|---|
| 1.0583 | N. F. VII | .. | 0.9660 | 91.30 |
| 1.0959 | N. F. VII | .. | 1.0135 | 92.45 |
| 1.0505 | N. F. VII | .. | 0.9840 | 93.63 |
| 1.0633 | N. F. VII | .. | 1.0115 | 95.15 |
| 1.1489 | N. F. VII | .. | 1.1210 | 97.57 |
| 1.1297 | N. F. VII | .. | 1.0840 | 95.93 |
| 1.0947 | N. F. VII | .. | 1.0700 | 97.70 |
| 1.2320 | N. F. VII | .. | 1.2135 | 98.50 |
| 1.0499 | N. F. VII | 30 | 1.0192 | 97.03 |
| 1.0785 | N. F. VII | 30 | 1.0670 | 98.77 |
| 2.4035 | N. F. VII | 30 | 2.2852 | 95.08 |
| 1.0152 | N. F. VII | 30 | 0.9614 | 94.67 |
| 0.9258 | N. F. VII | 30 | 0.8938 | 96.49 |
| 0.9537 | N. F. VII | 30 | 0.9295 | 97.46 |
| 0.6272 | N. F. VIII | 30 | 0.6289 | 100.30 |
| 0.6016 | N. F. VIII | 30 | 0.6031 | 100.30 |
| 0.7460 | N. F. VIII | 30 | 0.7471 | 100.20 |

TABLE III.—DETERMINATION OF CINNAMALDEHYDE BY N. F. VIII METHOD

| Weight of Sample, Gm. | Method | Period of Standing, Min. | Weight of Cinnamaldehyde Recovered, Gm. | Cinnamaldehyde Recovered, % |
|---|---|---|---|---|
| 1.0009 | N. F. VIII | 10 | 0.9945 | 99.36 |
| 1.0005 | N. F. VIII | 10 | 0.9945 | 99.40 |
| 1.0007 | N. F. VIII | 10 | 0.9981 | 99.74 |
| 1.0000 | N. F. VIII | 10 | 0.9893 | 98.93 |
| 1.0005 | N. F. VIII | 10 | 0.9841 | 98.36 |
| 1.0007 | N. F. VIII | 10 | 0.9781 | 97.74 |
| 1.0110 | N. F. VIII | 10 | 0.9819 | 97.12 |
| 1.0045 | N. F. VIII | 10 | 0.9766 | 97.22 |
| 1.0265 | N. F. VIII | 10 | 1.0048 | 97.89 |
| 1.0050 | N. F. VIII | 30 | 0.9863 | 98.14 |
| 1.0008 | N. F. VIII | 30 | 0.9833 | 98.25 |
| 1.0059 | N. F. VIII | 30 | 0.9958 | 99.00 |
| 1.0075 | N. F. VIII | 40 | 0.9996 | 99.22 |
| 1.0045 | N. F. VIII | 50 | 0.9967 | 99.22 |
| 1.0122 | N. F. VIII | 55 | 1.0086 | 99.64 |

TABLE IV.—DETERMINATION OF VANILLIN AND BENZALDEHYDE BY THE METHOD OF BRYANT AND SMITH (7)

| Compound | Weight of Sample, Gm. | Period of Standing, Min. | Weight of Compound Recovered, Gm. | Compound Recovered, % |
|---|---|---|---|---|
| Vanillin | 0.8979 | 30 | 0.8615 | 95.95 |
| | 0.9000 | 30 | 0.8345 | 92.72 |
| | 0.8989 | 30 | 0.8584 | 95.50 |
| | 0.9038 | 30 | 0.8837 | 97.78 |
| | 0.8999 | 30 | 0.8700 | 96.68 |
| | 0.9016 | 60 | 0.8806 | 97.68 |
| | 0.9005 | 60 | 0.8768 | 97.37 |
| Benzaldehyde | 0.5541 | 30 | 0.5367 | 96.85 |
| | 0.5546 | 30 | 0.5371 | 96.84 |
| | 0.7379 | 30 | 0.7152 | 96.93 |

and benzaldehyde were assayed by the method outlined in the monograph for Camphor Spirit in N. F. VIII (U. S. P. XII). This method proved grossly unsatisfactory because of the alcohol present and the precipitation of the reagent.

Iddles and Jackson developed a 2,4-dinitrophenylhydrazine method suitable for the determination of a series of representative and common aldehydes and ketones (8) and included vanillin and benzaldehyde in their study. In their method the 2,4-dinitrophenylhydrazine is dissolved in hydrochloric acid and the precipitation takes place at both room temperature and the temperature of an ice bath. The hydrazone is obtained on a filtering crucible and dried to constant weight at 80° (8).

TABLE V.—DETERMINATION OF BENZALDEHYDE AND VANILLIN BY THE METHOD OF IDDLES AND JACKSON (8)

| Compound | Temperature | Weight of Compound per 100 Cc., Gm. | Weight of Compound Recovered, Gm. | Compound Recovered, % |
|---|---|---|---|---|
| Benzaldehyde | Room | 0.2400 | 0.2273 | 94.74 |
| | Room | 0.2400 | 0.2267 | 94.44 |
| | Room | 0.2400 | 0.2271 | 94.64 |
| | Room | 0.2400 | 0.2271 | 94.64 |
| | Ice Bath | 0.2465 | 0.2321 | 94.15 |
| | Ice Bath | 0.2465 | 0.2316 | 93.95 |
| | Ice Bath | 0.2465 | 0.2318 | 94.05 |
| | Ice Bath | 0.2465 | 0.2323 | 94.25 |
| Vanillin | Ice Bath | 0.4008 | 0.3997 | 99.72 |
| | Ice Bath | 0.4008 | 0.4009 | 100.02 |
| | Ice Bath | 0.4008 | 0.3982 | 99.34 |

Vanillin and benzaldehyde were assayed by this method. From the data in Table V it is evident that the method gives a low recovery of benzaldehyde in our hands. Iddles and Jackson found the method satisfactory for benzaldehyde, the average recovery being 99.2%, but unsatisfactory for vanillin, the average recovery being 100.2% (8). In our laboratory study of the method, the method was found to give a recovery for vanillin approximating the theoretical value and that obtained in the methoxy determination.

## DISCUSSION

It was found that there is little difference between the N. F. VII and the N. F. VIII hydroxylamine methods. However, the N. F. VIII method seems easier and more efficient for an inexperienced laboratory worker. Care must be taken in titrating to the proper end point of *p*H of 3.45 and a greenish yellow color, and in matching the samples to the blank.

However, in the case of benzaldehyde there was a more marked difference between the two methods. The N. F. VII method gave low and inconsistent results, while the N. F. VIII method gave higher and concordant results. Although the length of time of standing was of little importance in the assay of vanillin, it was more significant in the assay of benzaldehyde and cinnamaldehyde.

No justification for the use of pyridine in the reagent could be found. The addition of pyridine does away with the need of titrating a blank but lowers the recovery of aldehyde.

The 2,4-dinitrophenylhydrazine method, was selected for study after the official method for Camphor Spirit in N. F. VIII was found to be unsatisfactory for vanillin and benzaldehyde. From a review of the available literature, the method suggested by Iddles and Jackson seemed to have given the most accurate results with these two compounds. Nevertheless, their proposed method gave a low recovery of benzaldehyde when the mixture was allowed to stand at either room temperature or in an ice bath. Although Iddles and Jackson obtained a high per cent of recovery in the assay of vanillin, the procedure, when studied in this laboratory, gave a recovery approximating the theoretical.

## SUMMARY

1. A study of various hydroxylamine volumetric methods for the determination of aldehydes was made, using vanillin, benzaldehyde, and cinnamaldehyde.

2. A comparison between the volumetric methods and gravimetric methods, using 2,4-dinitrophenylhydrazine as the precipitating agent, was made.

3. It was concluded that the method made official in N. F. VIII is most suitable for benzaldehyde and cinnamaldehyde.

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## Book Review

*Official and Tentative Methods of Analysis.* Volume VI. Edited by HENRY A. LEPPER. Association of Official Agricultural Chemists, Washington, D. C., 1945. xii + 932 pp. 76 figs. 23 x 15 cm. Price, $6.25.

This new and completely revised sixth edition of *Methods of Analysis, A. O. A. C.*, includes revised and new methods of the Association adopted during the five-year period since the fifth edition was published in 1940. For more than twenty years, *Methods of Analysis, A. O. A. C.* has been used as a reference and as a source of information on analytical methods, not only by agricultural chemists but by chemists in fields more or less remotely related to agricultural products. Many of the methods described in chapters, other than the one on drugs, may be applied, either directly or with slight modification, to pharmaceutical products. The appearance of a new edition is, accordingly, of especial interest to members of the pharmaceutical profession who apply analytical procedures to drugs.

The chapter on drugs, like nearly every other chapter in the book, has undergone noticeable revision, and a number of new official and tentative analytical methods have been added. Among the additions to this chapter, for example, are tentative methods for the determination of the individual bromides in Three Bromides Elixir and Five Bromides Elixir.

New chapters on Cosmetics; Enzymes; Gelatin, Dessert Preparations, and Mixes; Vitamins; and Extraneous Materials in Foods and Drugs have been added. Nearly all of the new chapters are of pharmaceutical interest. The chapter on Cosmetics presents tentative methods for the determination of aluminum and zinc in deodorants and anti-perspirants, an official method for the determination of sulfides in depilatory powders, and the analysis of hair preparations for 2,5-diaminotoluene, paraphenylenediamine, and salicylic acid. The chapter on Enzymes deals only with a method for determining the proteolytic activity of papain by measuring its digestive activity on casein.

The new chapter on Extraneous Materials in Foods and Drugs is of especial pharmaceutical interest. Section 501 (*a*) (1) of the Food, Drug, and Cosmetic Act provides that a drug is adulterated "if it has been prepared, packed, or held under insanitary conditions whereby it may have been contaminated with filth or whereby it may have been rendered injurious to health." Because of the many and varied factors to be considered in connection with this section, it is impossible for the official compendia to establish definite tolerances for certain types of extraneous material in drugs. Both the U. S. P. and the N. F., however, state: "Vegetable and animal drugs are to be as free as practicable from molds, insects, and other animal contamination, and from animal excreta," but no methods have been officially adopted for the determination of such contamination. Because of this, the inclusion of tentative methods for the determination of certain extraneous material in crude drugs is of especial interest. The procedures described have previously been available, but their publication in *Methods of Analysis* is highly desirable.

The methods included in the new edition are those in effect on the date of publication. They are subject to revision, and reprints of any changes adopted previous to the publication of the seventh edition will be sent to purchasers of the new sixth edition who fill out and mail to the Association a form provided with each copy of the book. The style and arrangement of the new edition are the same as in the fifth edition. The book, as in the past, is well documented and has a fairly complete and usable index. The paper and binding are of high quality and reflect the care that has gone into the new edition, and enhance the favorable impression of the work.—JUSTIN L. POWERS.